Original Article

Anti-Cancer Drugs Effective in Retinoblastoma: Based on a Protein-Protein Interaction Network

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Abstract

Background: This paper investigates the effects of potential drugs on differentially expressed genes (DEGs) associated with substantial alterations in retinoblastoma malignancy.

Material and Methods: The GSE125903 dataset consisting of ten samples was used in this study (seven cancer patients and three control samples). The genes were ordered according to their adjusted p value, and 2000 top differential expressed genes with adj p values less than 0.01 were chosen as statistically significant. The STRING database version 11.0 was used to display the interaction among genes. The Cytoscape3.8.2 and the Clusterviz plugin software were used to construct the modules for the PPI network, and five clusters of genes were formed. The DGIdb v4.2.0 database was used to study drug-gene interactions and identify potentially beneficial medicines for retinoblastoma malignancy. The DAVID v.6.8 database was used to study gene ontology (GO) and important biological pathways.

Results: CISPLATIN, TAMOXIFEN, and CYCLOPHOSPHAMIDE are the medicines that have been shown to be successful in treating retinoblastoma in our study. Additionally, we conducted a research on three other drugs: GEMCITABINE, OLAPARIB, and MITOXANTRONE. Although it is used to treat other diseases, it seems to have no apparent effects on retinoblastoma cancer treatment.

Conclusion: CISPLATIN, a drug that causes apoptosis in tumors, has been proven to be the most effective therapy for retinoblastoma and should be included in treatment regimens for this illness. Of course, we obtained this information based on bioinformatics techniques, and more clinical trials are needed for more reliable results.

Keywords: Protein-Protein Interaction Network; Retinoblastoma; Anti-Cancer.

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Introduction

Retinoblastoma is a common childhood cancer that begins with RB1 gene mutation [1]. This illness encompasses 3 % of childhood cancers and is the most common intraocular malignancy in children. Some believe that Retinoblastoma can be fatal if left untreated [2]. The discovery of the RB1 gene on the chromosome13q14 in the 1980s reported RB1 as the first tumor-suppressor gene [1].

Conditional loss of an allele of RB1 gene predisposes a person to cancer, and loss of another allele from the developing retinal cell triggers the onset of a retinoblastoma tumor [1]. RB codes a nuclear phosphoprotein that regulates retinoblastoma proliferation and development [3]. Loss of tumor suppressor functions in RB protein leads to out-of-control cell division and frequent genomic changes during tumor progression [4].

Systems biology approach successfully helps researchers to investigate molecular mechanisms of inhibition and activation of the genes and transcription factors, and detect novel molecular biomarkers of phenotypes [5-9]. Many RNA-RNA, RNA-miRNA, and RNA-Lnc-RNA interaction beside other macromolecules interactions in the cells; have been detected in the light of systems biology method [10-13]. Recently, additional mechanisms leading to retinoblastoma have been investigated, including genomic instability, defects in the DNA mismatch repair system, DNA methylation changes, amplification, histone acetylation / distillation, and aneuploidy [14].

About 60 % of patients with unilateral retinoblastoma are often sporadic disease and have no family history. In some patients, multiple tumor foci can be diagnosed (unilateral multifocal retinoblastoma). About 40 % of patients have bilateral retinoblastoma. Most of these patients have more than one focus in their eyes (bilateral multi focal retinoblastoma) [15]. In general, children with bilateral retinoblastoma are diagnosed earlier than those with unilateral retinoblastoma [15]. Retinoblastoma is first diagnosed when a white tumor is seen through the eye pupil (known as leukocoria) or when it blocks vision [1]. Early diagnosis and referral to medical centers can significantly reduce mortality [16].

The disease has no specific area for spread. The highest prevalence is found among populations with high birth rates, such as Asia and Africa [1]. In the United States and Europe, the prevalence of retinoblastoma is about 11 cases per million people under the age of 5. Out of every 15,000 to 20,000 healthy births, there are about 9,000 new cases worldwide, and about 200 occur in the United States [17].

The highest mortality rate (up to 70 %) is in less developed regions, such as Asia and Africa, while in Europe, the United States, and Canada it is 3 to 5 % [1].

Early diagnosis and prompt treatment can increase treating intraocular tumors by up to 95 %. For early diagnosis, it is essential for family members to be screened [18]. If retinoblastoma spreads outside the eye, the death risk is very high [19]. Some treatments for ocular rescue include intra-arterial chemotherapy, invasive focal therapy, and external beam radiation therapy [20].

This study establishes a network in order to identify possible medicines that may have an effect on Retinoblastoma. For this reason we find out the genes related to Retinoblastoma and reconstruct the protein-protein interaction network using the STRING database. Then, the modules are created using the Cytoscape software and the clusterviz plugin. In the next step, we find potential EDCs using the DGIDB database and create drug-gene network. Finally, we introduce a high degree drug in

this network. We hope these findings help researchers learn more about Retinoblastoma.

Material and Methods

Dataset

GSE125903 is a dataset of gene expression profiles acquired from the GEO database (http://www.ncbi.nlm.nih.gov/geo/). Then we used GEO2R tool for processing of raw data and remove outliers and normalization of samples and also find differential expressed genes. The top 2,000 differentially expressed genes with an adj P value less than 0.01 were chosen from the GSE125903 dataset, which comprised genes implicated in retinoblastoma malignancy. So, the DEGs selection procedure is based on adj P value. As a result, we rank the genes by adj P value and then choose the top 2000 differential expressed genes with adj P value less than 0.01.

Protein–Protein Interactions Network (PPIN)

Using the STRING database v.11.0, Top 2000 differentially expressed genes with adj P value less than 0.01 were used to create the protein-protein interaction network. The data were then displayed using the Cytoscape v.3.8.2. The ClusterViz analyzes clusters using a variety of techniques. The rapid agglomerate edge clustering (FAG-EC) technique was used for this study's objective.

STRING is an online tool for drawing biological networks especially protein-protein interaction network networks. Two different types of interactions including physical and functional interactions are available in this tool. In this study, 5 strong modules were created for the PPI network using Cytoscape3.8.2 and the fast agglomerate edge clustering (FAG-EC) technique. The following filters were used: In/OutThreshold: 20, Overlapped, Strong. Finally, five different PPI groups were obtained by the Clusterviz plugin. At first different numbers were used for threshold then we compare the modules of each threshold number then we select proper one.

Drug Gene Interaction (DGIdb)

Drug gene interaction data from the DGIdb v4.2.0 database was used for identified gene clusters (www.dgidb.org) [21] to find potential drugs effective in retinoblastoma cancer. The DGIdb includes more than 40,000 genes and 10,000 pharmaceuticals that are engaged in more than 100,000 drug-gene interactions or fall into one of 42 druggable gene categories. Users may input a list of genes to gain all known or druggable genes included in that list. The results may be filtered according to the source, interaction type, or gene category. DGIdb is a Ruby on Rails and PostgreSQL application that utilizes configurable relational database architecture to store information from a variety of sources.

Gene ontology analysis (GO)

The DAVID v.6.8 enrichment online tool (https://david.ncifcrf.gov/) [22] was used to examine important biological pathways. We used functional enrichment analysis to deduce the biological processes behind differentially expressed genes. The DAVID bioinformatics program was used to undertake functional enrichment of gene ontology (GO) and reactome pathway studies. For this purpose, the genes of 5 sub-networks, which we had previously obtained, were used.

David is a biological database with multiple data analytics that is used to extract biological significance from a huge gene/protein list in a systematic manner [22].

Using this method, we conducted gene

ontology and obtained useful information from biological processes (BP).

Results

Dataset

We used the GSE125903 dataset, which contained the genes involved in retinoblastoma cancer, and selected top 2,000 differential expressed genes with an adj-pvalue less than 0.01.

Our research included ten samples, seven of which were cancer samples, and three were control samples [Table1]. The genes were ordered according to their adjusted p values and 2000 top genes with adj-pvalue less than 0.01 were chosen as statistically significant. Supplementary file s1 contains information about the dataset.

Analyzing these genes reveals the number of up and down-regulated genes. According to fold changes, 792 genes are down-regulated, and 1208 genes are up-regulated. For down-regulated genes, we use a fold change less than 0 and for up-regulated genes, we use a factor change greater than 0.

Table 1: This table indicates the number of each group

Normal samples(healthy control)	Cancer samples	Expression Array:
7	3	GPL16791 Illumina HiSeq 2500 (Homo sapiens)

PPI analysis

The PPI network of top 2000 differential expressed genes was obtained from the STRING database [supplementary file S2]. Clustering analysis was used to identify highly interacting protein groups. Five protein modules were discovered. By drawing a PPI network, 637 nodes and 1130 edges were formed and entered into the cytoscope3.8.2 software. To obtain the most important protein networks, 5 sub-network were plotted, and 171 genes were obtained [table 2] [Figure 2] [supplementary file S3].

Gene ontology and pathway enrichment analysis

The DAVID enrichment online tool was used to investigate the biological roles of genes in gene clusters at a cellular level. Table2 indicates the top 15 different biological pathways with the most significant P value identified using gene ontology.

We mentioned top 15 pathways of biological processes (BP), and the genes of each cluster are classified in terms of a P value close to zero and an FDR index [table3]. Complete molecular function (MF) information is available in the [supplementary file S5].

Drug-gene interaction network

The outcome of a gene-drug interaction was visualized using the Cytoscape3.8.2 program, and the top six drugs with the highest effect on target genes were selected for further investigation [Figure3]. [supplementary file S4] contains the whole drug gene interaction network. The DGIdb v4.2.0 database was searched for drug-gene interactions. Six medications that had the largest influence on genes either as an inhibitor or as an antagonist were chosen with a significant effect on the genes. CISPLATIN - TAMOXIFEN - GEMCITABINE – OLAPARIB - MITOXANTRONE - CYCLOPHOSPHAMIDE. CISPLATIN with

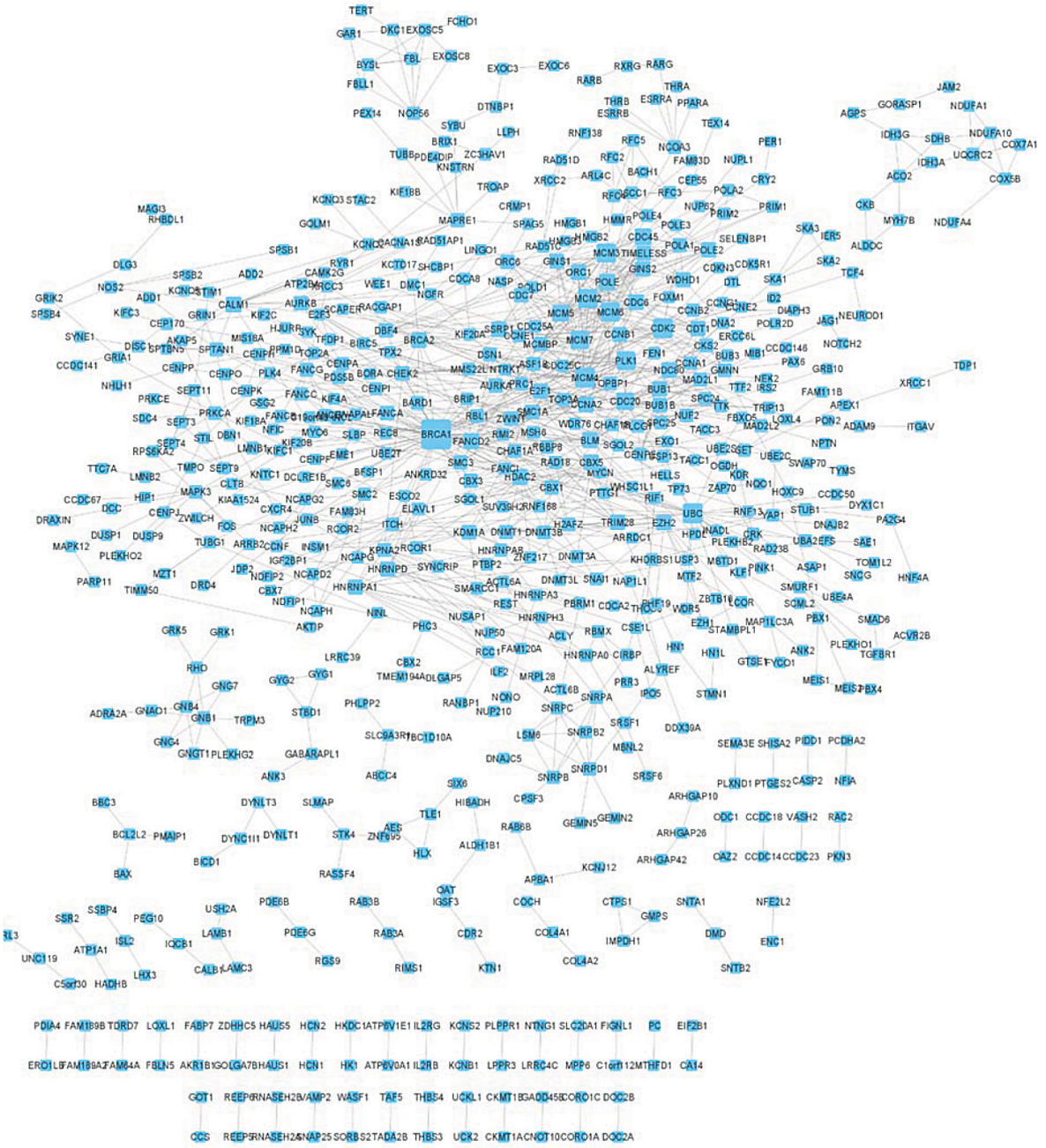


Figure 1: The entire PPI network consists of 637 nodes between proteins and 1130 edges consisting of physical interactions

degree 9 was the most important drug in the binary network.

Discussion

Our goal was to find effective drugs for retinoblastoma based on a protein network design and drug gene interaction.

From the obtained data, we selected 6 drugs that had the highest degree of interaction, including CISPLATIN,TAMOXIFEN,GEMCITABINE, OLAPARIB, MITOXANTRONE, and CYCLOPHOSPHAMIDE. These drugs can be used as the inhibitor or antagonist effective in

Table 2: List of each sub-networks and gene names

Cluster	Nodes	Edges	GENES
1	28	68	DNMT3A, REST, KDM1A, WHSC1L1, EZH2, SNAI1, HDAC2, DNMT1, ZBTB16, SUV39H2, CHAF1B, DNMT3B, CBX3, INSM1, CBX1, CHAF1A, SGOL1, PHF19, LCOR, MTF2, TRIM28, EZH1, ZNF217, RCOR2, RCOR1, CBX5, MYCN, DNMT3L
2	24	131	MMS22L, POLE, GINS1, MCM4, CDC7, GINS2, ORC6, SELENBP1, POLE2, MCM5, CDC45, ORC1, MCM3, TIMELESS, POLD1, MCM2, POLE4, CDC6, MCMBP, POLE3, WDHD1, MCM7, DBF4, MCM6
3	42	102	TACC1, CDC25A, CDKN3, PLK1, DIAPH3, BUB1B, CDC20, CDC25C, FOXM1, ZWINT, AURKA, BFSP1, CDK5R1, LOXL4, WEE1, SCAPER, CCNA2, CDK2, TRIP13, BUB3, CCNB1, UBE2S, SPC24, CKS2, CDT1, FBXO5, TTK, CCNA1, MAD2L1, SPC25, CCNG1, NUF2, CENPE, NDC80, CCNE2, BUB1, CCNE1, DTL, TACC3, DSN1, BORA, CCNB2
4	48	87	BLM, FANCI, DNAJB2, SMC6, UBC, KIAA1524, BRCA1, RAD18, FANCC, C19orf40, ANKRD32, TTF2, TOM1L2, NFIC, KIFC1, UBE4A, PLEKHB2, KIF18A, FANCL, UBA2, RAD23B, BRIP1, FANCB, PRC1, PINK1, EME1, FAM83H, FANCE, FANCG, CCDC50, TOP3A, KLF1, KIF4A, FANCA, SAE1, UBE2T, RNF13, BARD1, KIF20B, DCLRE1B, ERCC6L, CENPF, RMI2, FEN1, POLR2D, FANCD2, SNCG, TOPBP1
5	29	30	SPTAN1, NDFIP2, NDFIP1, ATP2B4, IRS2, GRB10, NTRK1, KDR, PLCG1, EFS, LINGO1, RYR1, GRIN1, CRK, ASAP1, SPTBN5, SYK, YAP1, ARRDC1, KCNQ5, ITCH, CAMK2G, CALM1, NGFR, INADL, STAC2, CACNA1S, DBN1, CXCR4

the retinoblastoma disease.

A study conducted in 1998 showed that CISPLATIN is a drug that stimulates apoptosis in Y79 cells. Human Y79 cells are a line of retinoblastoma cells derived from a multi potential stem cell of the neural retina [23]. It has also been shown that treatment with CISPLATIN and carboplatin increases p53 and p21 levels and decreases Bcl-2 [23].

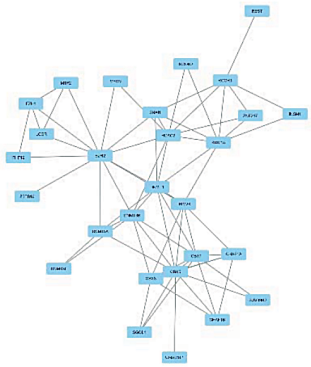
It has also been shown that cluster in proteins can have an anti-apoptotic effect on CISPLATIN-induced apoptosis and prevent cell death. Cluster is a protective protein of various retinal cells [24].

A study on animals also found that treatment with calcitriol and CISPLATIN reduced tumors by 65% compared with controls [25].

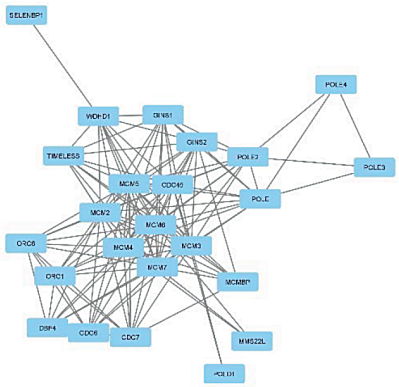
TAMOXIFEN is a widely used drug for treating breast cancer. However, previous evidence suggests that this drug may be effective in treating metastatic retinoblastoma, which improves life length and quality in patients [26].

A study of two cases confirmed the therapeutic effect of vincristine and CYCLOPHOSPHAMIDE on retinoblastoma tumors and objective tumor regression [27].

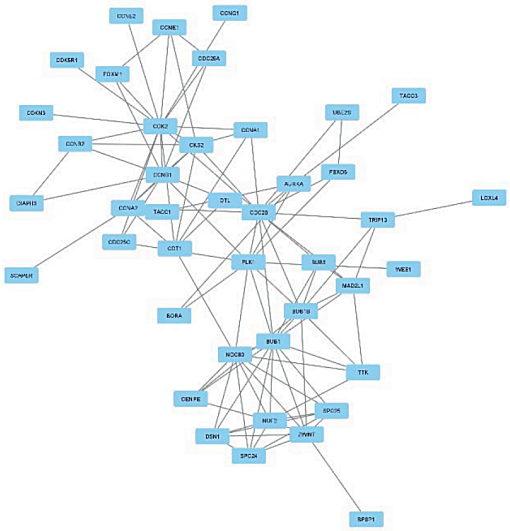
Module 1



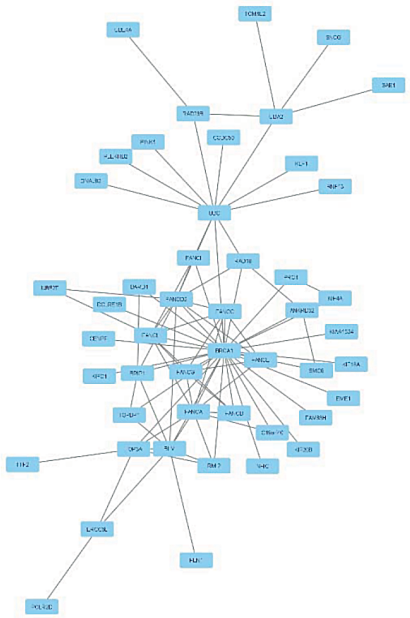
Module 2



Module 3



Module 4



Module 5

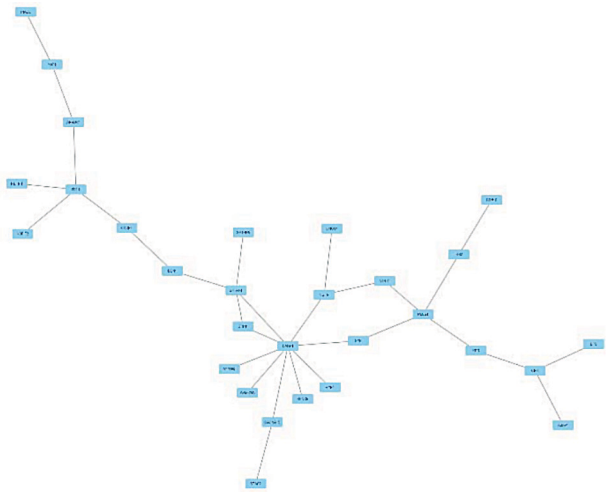


Figure 2: Five modules obtained from the whole PPI network

Table 3: Top 15 different biological pathways with the most significant P values identified using gene ontology in DAVID v.6.8 Database

biological processes	P Value	FDR	Gene list
GO:0022402~cell cycle process	3.61E-45	1.10E-41	"DCLRE1B, ERCC6L, FEN1, MCM7, BUB1B, BRCA1, FOXM1, CDC20, EME1, NUF2, FBXO5, TOPBP1, POLE, BORA, TOP3A, CDC25C, CDC25A, CCNA2, TOM1L2, CCNA1, DBF4, CCNE2, FANCD2, CCNE1, KIFC1, CKS2, MCM3, TIMELESS, MCM4, MCM5, MCM6, KIF20B, DTL, MCM2, BLM, TTK, AURKA, CCNB2, BRIP1, DSN1, CCNB1, ORC6, CDC45, ORC1, UBC, MCMBP, BUB3, CAMK2G, BUB1, FANCI, BARD1, GINS1, GINS2, CDT1, PLK1, ATP2B4, FANCA, CDC7, CDC6, FANCG, NDC80, ZWINT, CENPE, KIF18A, CENPF, WEE1, UBE2S, PRC1, POLE2, KIF4A, CCNG1, CDK2, TACC3, INSM1, TRIP13, CALM1, SPC24, CDKN3, CDK5R1, SPC25, EZH2, MAD2L1"
GO:0007049~cell cycle	2.24E-44	3.41E-41	"DCLRE1B, SUV39H2, ERCC6L, FEN1, MCM7, BUB1B, BRCA1, FOXM1, CDC20, CHAF1B, CHAF1A, EME1, NUF2, FBXO5, TOPBP1, POLE, BORA, TOP3A, DNMT3A, CDC25C, CDC25A, CCNA2, TOM1L2, CCNA1, DBF4, CCNE2, FANCD2, CCNE1, KIFC1, CKS2, MCM3, TIMELESS, MCM4, MCM5, MCM6, KIF20B, DTL, MCM2, BLM, TTK, AURKA, CCNB2, BRIP1, DSN1, CCNB1, ORC6, CDC45, ORC1, UBC, MCMBP, BUB3, CAMK2G, BUB1, FANCI, BARD1, GINS1, GINS2, CDT1, PLK1, ATP2B4, FANCA, CDC7, CDC6, FANCG, NDC80, ZWINT, CENPE, KIF18A, CENPF, WEE1, UBE2S, PRC1, POLE2, KIF4A, CCNG1, CDK2, TACC3, TACC1, INSM1, TRIP13, CALM1, SAE1, SPC24, CDKN3, CDK5R1, SPC25, EZH2, MAD2L1"
GO:1903047~mitotic cell cycle process	1.29E-42	1.31E-39	"ERCC6L, MCM7, BUB1B, FOXM1, CDC20, EME1, NUF2, FBXO5, TOPBP1, POLE, BORA, CDC25C, CDC25A, CCNA2, TOM1L2, CCNA1, DBF4, CCNE2, CCNE1, KIFC1, CKS2, MCM3, TIMELESS, MCM4, MCM5, MCM6, KIF20B, MCM2, BLM, TTK, AURKA, CCNB2, DSN1, CCNB1, ORC6, CDC45, ORC1, UBC, MCMBP, BUB3, CAMK2G, BUB1, FANCI, GINS1, GINS2, CDT1, PLK1, CDC7, CDC6, NDC80, ZWINT, CENPE, KIF18A, CENPF, WEE1, UBE2S, PRC1, POLE2, KIF4A, CCNG1, CDK2, TACC3, CALM1, SPC24, CDKN3, SPC25, EZH2, MAD2L1

biological processes	P Value	FDR	Gene list
GO:0000278~mitotic cell cycle	2.24E-41	1.70E-38	"ERCC6L, MCM7, BUB1B, FOXM1, CDC20, EME1, NUF2, FBXO5, TOPBP1, POLE, BORA, DNMT3A, CDC25C, CDC25A, CCNA2, TOM1L2, CCNA1, DBF4, CCNE2, CCNE1, KIFC1, CKS2, MCM3, TIMELESS, MCM4, MCM5, MCM6, KIF20B, MCM2, BLM, TTK, AURKA, CCNB2, DSN1, CCNB1, ORC6, CDC45, ORC1, UBC, MCMBP, BUB3, CAMK2G, BUB1, FANCI, GINS1, GINS2, CDT1, PLK1, CDC7, CDC6, NDC80, ZWINT, CENPE, KIF18A, CENPF, WEE1, UBE2S, PRC1, POLE2, KIF4A, CCNG1, CDK2, TACC3, CALM1, SPC24, CDKN3, SPC25, EZH2, MAD2L1"
GO:0006259~DNA metabolic process	1.85E-38	1.12E-35	"DCLRE1B, FEN1, MCM7, KDM1A, BRCA1, SMC6, FOXM1, CHAF1B, DNMT3L, CHAF1A, EME1, TRIM28, DNMT3B, TOPBP1, POLE, RMI2, TOP3A, DNMT3A, CDC25C, RAD23B, CDC25A, MMS22L, DBF4, CCNE2, FANCD2, CCNE1, MCM3, TIMELESS, MCM4, MCM5, MCM6, DTL, MCM2, BLM, DNMT1, BRIP1, ORC6, CDC45, ORC1, POLD1, UBC, POLR2D, MCMBP, FANCI, BARD1, GINS1, GINS2, CDT1, NDFIP1, FANCL, FANCA, FANCC, CDC7, FANCB, CDC6, FANCE, FANCG, POLE4, CENPF, UBE2T, NFIC, POLE2, POLE3, CDK2, TRIP13, RAD18, EZH2"
GO:0044770~cell cycle phase transition	3.11E-36	1.58E-33	"MCM7, BUB1B, FOXM1, CDC20, FBXO5, TOPBP1, POLE, BORA, CDC25C, CDC25A, CCNA2, CCNA1, DBF4, CCNE2, CCNE1, CKS2, MCM3, TIMELESS, MCM4, MCM5, MCM6, MCM2, BLM, TTK, AURKA, CCNB2, CCNB1, ORC6, CDC45, ORC1, UBC, BUB3, CAMK2G, BUB1, FANCI, CDT1, PLK1, ATP2B4, CDC7, CDC6, NDC80, CENPE, CENPF, WEE1, UBE2S, POLE2, CDK2, TACC3, CALM1, CDKN3, EZH2, MAD2L1"
GO:0051276~chromosome organization	8.39E-36	3.65E-33	"DCLRE1B, SUV39H2, ERCC6L, FEN1, MCM7, KDM1A, BUB1B, BRCA1, SMC6, CDC20, CHAF1B, CHAF1A, TRIM28, NUF2, DNMT3B, POLE, WHSC1L1, TOP3A, DNMT3A, RAD23B, FANCD2, KIFC1, MTF2, MCM3, MCM4, MCM5, MCM6, MCM2, BLM, DNMT1, HDAC2, TTK, AURKA, BRIP1, DSN1, CCNB1, CDC45, POLD1, UBC, MCMBP, BUB3, BUB1, GINS1, GINS2, CBX3, PLK1, CDC6, NDC80, ZWINT, POLE4, CENPE, KIF18A, CENPF, REST, PINK1, PRC1, POLE2, KIF4A, POLE3, CDK2, TACC3, TRIP13, SPC24, RCOR1, EZH1, PHF19, SPC25, EZH2, MAD2L1"

biological processes	P Value	FDR	Gene list
GO:0044772~mitotic cell cycle phase transition	3.34E-35	1.27E-32	"MCM7, BUB1B, FOXM1, CDC20, FBXO5, TOPBP1, POLE, BORA, CDC25C, CDC25A, CCNA2, CCNA1, DBF4, CCNE2, CCNE1, CKS2, MCM3, MCM4, MCM5, MCM6, MCM2, BLM, TTK, AURKA, CCNB2, CCNB1, ORC6, CDC45, ORC1, UBC, BUB3, CAMK2G, BUB1, FANCI, CDT1, PLK1, CDC7, CDC6, NDC80, CENPE, CENPF, WEE1, UBE2S, POLE2, CDK2, TACC3, CALM1, CDKN3, EZH2, MAD2L1"
GO:0006260~DNA replication	1.29E-33	4.36E-31	"BLM, FEN1, MCM7, BRCA1, CHAF1B, BRIP1, ORC6, CHAF1A, CDC45, ORC1, EME1, POLD1, MCMBP, TOPBP1, POLE, BARD1, GINS1, GINS2, CDT1, RMI2, TOP3A, CDC7, CDC6, CDC25C, CDC25A, MMS22L, DBF4, CCNE2, CCNE1, NFIC, POLE2, POLE3, CDK2, MCM3, TIMELESS, MCM4, MCM5, MCM6, DTL, MCM2"
GO:0048285~organelle fission	4.20E-28	1.28E-25	"ERCC6L, BUB1B, TTK, AURKA, CDC20, CCNB2, DSN1, CCNB1, EME1, NUF2, KDR, MCMBP, FBXO5, BUB3, BUB1, BORA, PLK1, TOP3A, FANCA, CDC6, CDC25C, CDC25A, NDC80, ZWINT, CCNA2, TOM1L2, CCNA1, CENPE, KIF18A, CENPF, WEE1, PINK1, FANCD2, UBE2S, PRC1, KIFC1, KIF4A, CCNG1, CDK2, CKS2, TIMELESS, TACC3, TRIP13, KIF20B, SPC24, MAD2L1, SPC25"
GO:0051301~cell division	1.87E-27	5.18E-25	"BLM, ERCC6L, BUB1B, AURKA, CDC20, CCNB2, DSN1, CCNB1, NUF2, MCMBP, FBXO5, BUB3, BUB1, BORA, ZBTB16, PLK1, CDC7, CDC6, CDC25C, CDC25A, NDC80, ZWINT, CCNA2, CCNA1, CENPE, CENPF, WEE1, CCNE2, CCNE1, UBE2S, PRC1, KIFC1, KIF4A, CCNG1, CDK2, CKS2, TIMELESS, TACC3, MCM5, TACC1, CALM1, KIF20B, SPC24, MAD2L1, SPC25"
GO:0000280~nuclear division	3.32E-27	8.42E-25	"ERCC6L, BUB1B, TTK, AURKA, CDC20, CCNB2, DSN1, CCNB1, EME1, NUF2, MCMBP, FBXO5, BUB3, BUB1, BORA, PLK1, TOP3A, FANCA, CDC6, CDC25C, CDC25A, NDC80, ZWINT, CCNA2, TOM1L2, CCNA1, CENPE, KIF18A, CENPF, WEE1, FANCD2, UBE2S, PRC1, KIFC1, KIF4A, CCNG1, CDK2, CKS2, TIMELESS, TACC3, TRIP13, KIF20B, SPC24, MAD2L1, SPC25"

biological processes	P Value	FDR	Gene list
GO:0007067~mitotic nuclear division	7.24E-26	1.70E-23	"ERCC6L, BUB1B, TTK, AURKA, CDC20, CCNB2, DSN1, CCNB1, NUF2, MCMBP, FBXO5, BUB3, BUB1, BORA, PLK1, CDC6, CDC25C, CDC25A, NDC80, ZWINT, CCNA2, TOM1L2, CCNA1, CENPE, KIF18A, CENPF, WEE1, UBE2S, PRC1, KIFC1, KIF4A, CCNG1, CDK2, TIMELESS, TACC3, KIF20B, SPC24, MAD2L1, SPC25"
GO:0006270~DNA replication initiation	9.94E-26	2.16E-23	"GINS2, MCM7, CDC7, CDC6, ORC6, CDC45, ORC1, CCNE2, CCNE1, POLE2, CDK2, MCM3, MCM4, MCM5, TOPBP1, MCM6, POLE, MCM2"
GO:0006261~DNA-dependent DNA replication	1.99E-24	4.05E-22	"BLM, FEN1, MCM7, ORC6, CDC45, ORC1, EME1, POLD1, MCMBP, TOPBP1, POLE, GINS1, GINS2, CDT1, CDC7, CDC6, MMS22L, CCNE2, CCNE1, POLE2, CDK2, MCM3, MCM4, MCM5, MCM6, MCM2"

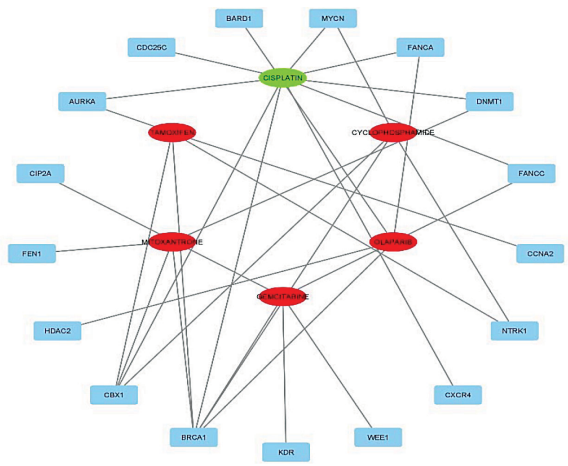


Figure 3: A high-degree drug-gene network based on six drugs. Genes are represented by blue rectangles, whereas medications are represented by red ovals. The most significant medication in the binary network, which is indicated in green, is CISPLATIN with a degree of 9

CYCLOPHOSPHAMIDE is known to be the most effective drug in treating a human retinoblastoma xenograft model, which was directly transplanted into the anterior chamber of the naked mouse eye [28].

A study in 2012 showed that the four-drug chemotherapy protocol, including CISPLATIN, Etoposide, Vincristine, and CYCLOPHOSPHAMIDE, had satisfactory results in treating retinoblastoma patients [29].

It seems that retinoblastoma treatment with CYCLOPHOSPHAMIDE may be associated with an increased risk of leiomyosarcoma in the bladder. The results of our study confirm this finding.

Several studies reported the following results: A case of leiomyosarcoma in the bladder was reported in an 18-year-old boy who had been treated with CYCLOPHOSPHAMIDE of retinoblastoma for 6 years and was diagnosed 50 days after birth [30].

Another case is a 22-year-old man with high-grade leiomyosarcoma. The patient's previous medical history was remarkable. Retinoblastoma was diagnosed at birth for the patient who underwent surgery with adjuvant chemotherapy with CYCLOPHOSPHAMIDE and vincristine 9 months after birth [31].

Another study diagnosed bladder

leiomyosarcoma in a 22-year-old woman who had been treated with CYCLOPHOSPHAMIDE of retinoblastoma for 68 months in 18-months postpartum [32].

GEMCITABINE has been used in treating advanced non-small cell lung cancer (NSCLC) and has generally improved a wide range of symptoms and general functioning [33].

OLAPARIB is also a PARP inhibitor approved by the Food and Drug Administration(FDA) in 2014 for the treatment of recurrent ovarian cancer in BRCA1/2-mutated women [34].

MITOXANTRONE is a drug approved by the FDA in 1987 for treating acute adult myeloid leukemia, in 1996 for symptomatic hormone-resistant prostate cancer, and in 2000 for worsening relapsing-remitting multiple sclerosis (MS), secondary progressive MS, and progressive-relapsing MS [35].

In our study, it seems that GEMCITABINE, OLAPARIB, and MITOXANTRONE do not have a credible effect on treating f retinoblastoma cancer and are used in other diseases.

Conclusions

This study intended to discover drugs that can be effective in treating retinoblastoma. To achieve this goal, we used bioinformatic tools and techniques. To find the main retinoblastoma genes, we plotted the protein-protein network for 2,000 genes. The network was constructed using the PPI modules and the drug-gene interactions. It seems that the drug compounds obtained in our research play an important role in the disease treatment. Our study is conducted based on computational methods. Thus, it is necessary to conduct in vitro and in vivo clinical trials in the future. We hope that the results help improve current treatment processes.

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Footnotes and Financial Disclosures

Conflict of interest

The authors have no conflict of interest with the subject matter of the present manuscript.