

# Bioinformatics analysis of the CDK2 functions in neuroblastoma

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**Abstract.** The present study aimed to elucidate the potential mechanism of cyclin-dependent kinase 2 (CDK2) in neuroblastoma progression and to identify the candidate genes associated with neuroblastoma with CDK2 silencing. The microarray data of GSE16480 were obtained from the gene expression omnibus database. This dataset contained 15 samples: Neuroblastoma cell line IMR32 transfected with CDK2 shRNA at 0, 8, 24, 48 and 72 h (n=3 per group; total=15). Significant clusters associated with differentially expressed genes (DEGs) were identified using fuzzy C-Means algorithm in the Mfuzz package. Gene ontology and pathway enrichment analysis of DEGs in each cluster were performed, and a protein-protein interaction (PPI) network was constructed. Additionally, functional annotation of DEGs in clusters was performed for the detection of transcription factors and tumor-associated genes. A total of 4 clusters with significant change tendency and 1,683 DEGs were identified. The hub nodes of the PPI network constructed by DEGs in Cluster 1, Cluster 2, Cluster 3 and Cluster 4 were MDM2 oncogene, E3 ubiquitin protein ligase (*MDM2*), cyclin-dependent kinase 1 (*CDK1*), proteasome (prosome, macropain) 26S subunit, non-ATPase, 14 (*PSMD14*) and translocator protein (18 kDa) (*TSPO*) respectively. These genes were significantly enriched in the p53 signaling pathway, cell cycle, proteasome and systemic lupus erythematosus pathways. *MDM2*, *CDK1*, *PSMD14* and *TSPO* may be key target genes of CDK2. CDK2 may have key functions in neuroblastoma progression by regulating the expression of these genes.

## Introduction

Neuroblastoma is an embryonal tumor that arises from the sympathetic nervous system, accounts for ~15% of

childhood cancer mortality (1,2). Despite intensive myeloablative chemotherapy, survival rates for neuroblastoma have not substantively improved; relapse is common and frequently leads to mortality (3,4). Like most human cancers, this childhood cancer can be inherited; however, the genetic aetiology remains to be elucidated (3). Therefore, an improved understanding of the genetics and biology of neuroblastoma may contribute to further cancer therapies.

In terms of genetics, neuroblastoma tumors from patients with aggressive phenotypes often exhibit significant MYCN proto-oncogene, bHLH transcription factor (*MYCN*) amplification and are strongly associated with a poor prognosis (5). MYCN, a member of MYC proto-oncogene family, functions as a transcriptional factor, which controls cell growth and proliferation and thus has an important role in driving tumorigenesis of neuroblastoma cells (6,7). Additionally, the overexpression of MYC genes in non-MYC-amplified cells may induce apoptosis (8). A previous study by Molenaar *et al* (9) confirmed that inactivation of cyclin-dependent kinase 2 (*CDK2*) was synthetically lethal to neuroblastoma cells with MYCN-amplification and overexpression (9). The *CDK2* gene encodes a protein that is member of serine/threonine protein kinase family that is involved in cell cycle regulation (10). Additionally, *CDK2* has been demonstrated to regulate the progression through the cell cycle (11). A previous study also has determined that the targeting of aberrant cell cycle checkpoints in cancer cells may inhibit tumor growth and induce cell death (12). *CDK2* is a vital regulator of S-phase progression and is deemed to be an anticancer drug target (9,13). Additionally, *CDK2* inhibitors may act as potential MYCN-selective cancer therapeutics in the treatment of neuroblastoma (9). However, the molecular mechanism of *CDK2* in the genesis of childhood cancer neuroblastoma remains to be fully elucidated.

In a previous study, microarray data from GSE16480 was used for identification of the upregulated genes following *CDK2* silencing. The findings revealed that these upregulated genes were target genes of p53, and silencing of p53 protected the cells from MYCN-driven apoptosis (9). However, to the best of our knowledge, there was no systematic and comprehensive analysis for this expression profile. The present study downloaded the microarray data of GSE16480 and then identified significant clusters associated with differentially expressed genes (DEGs) following *CDK2* silencing. Gene ontology (GO) and pathway enrichment analysis of DEGs in each cluster were performed, and protein-protein interaction

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(PPI) network was constructed. Additionally, a functional annotation of DEGs in the clusters was performed. The present study aimed to identify key genes and biological pathways underlying the progression of neuroblastoma with CDK2 silencing by means of comprehensive bioinformatics analysis to further elucidate the function of CDK2 in neuroblastoma progression and determine potential targets for future cancer therapies.

## Materials and methods

**Source of data.** The microarray data GSE16480 was downloaded from Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>) database based on the platform of GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array), which was deposited by Molenaar *et al* (9). This dataset contained 15 samples: Neuroblastoma cell line IMR32 was transfected with CDK2 shRNA at 0, 8, 24, 48 and 72 h (n=3 per group; total=15).

**Data preprocessing.** Background correction, quartile normalization and probe summarization were performed to normalize the gene expression intensities obtained from the raw dataset using robust multi-array average algorithm (14), and the gene expression time-course matrix of samples was acquired.

**Soft clustering analysis.** Noise robust soft clustering of gene expression time-course data was implemented using the fuzzy C-Means algorithm (15) in the Mfuzz package (15,16). The following parameters were set: Minimum Standard Deviation=0.4, score=0.7. This method may assign genes into several clusters according to the expression pattern of DEGs. Then, clusters with significant change tendency were screened for further analysis.

**PPI network construction.** The Search Tool for the Retrieval of Interacting Genes (STRING) database (17) is a database for the exploration and analysis of known and predicted protein interactions, including both experimental and predicted interaction information. The present study used the STRING online tool to analyze the PPIs of up and downregulated genes with required confidence (combined score) >0.4. The hub proteins were subsequently identified from the PPI network based on connectivity degree analysis.

**GO and pathway enrichment analysis.** GO (18) is widely used for the studies of large-scale genomic or transcriptomic data in function. Kyoto Encyclopedia of Genes and Genomes (KEGG) (19) is an online pathway database, which deals with genomes, enzymatic pathways and biological chemicals. The present study identified over-represented GO categories in biological processes and significant KEGG pathways of the DEGs in each cluster. The P-value of the default hypergeometric test of >0.05 was selected as the threshold.

**Functional annotation of DEGs in each cluster.** The tumor suppressor gene (TSGene) (20) database provides detailed annotation for each tumor suppressor gene (TSGs), such as transcription factors (TF) regulations. The tumor-associated

gene (TAG) database (21) summarizes attributes for a specific entity associated with the TAGs.

Functional annotations of DEGs in clusters were performed for the detection of TFs and TAGs and both databases, TSGene and TAG database, were used to identify oncogenes and tumor suppressor genes.

## Results

**Soft clustering analysis.** Soft clustering analysis of gene expression time-course data identified 4 clusters with significant change tendency (Fig. 1). Cluster 1 presented an increasing trend (Fig. 1A). Specifically, the expression levels of genes exhibited an increase from 0 to 8 h; subsequently the levels increased significantly from 8 to 48 h and remained constant from 48 to 72 h. It is of note that the change tendency of gene expression in Cluster 3 (Fig. 1C) at different time points is evidently opposite to those observed in Cluster 1. The expression levels of genes in Cluster 3 decreased slightly from 0 to 8 h, subsequently the levels decreased significantly from 8 to 48 h and remained constant from 48 to 72 h. In addition, the change tendency of gene expression in Cluster 2 (Fig. 1B) at different time points was evidently opposite to those observed in Cluster 4 (Fig. 1D). The expression levels of genes in Cluster 2 decreased from 0 to 24 h and subsequently decreased significantly from 24 to 72 h, whereas in Cluster 4 this trend was reversed.

Additionally, DEGs with the same expression pattern as change tendency of clusters was screened. A total of 1,683 DEGs were identified, including 337 upregulated genes in Cluster 1, 649 downregulated genes in Cluster 2, 387 downregulated genes in Cluster 3, and 387 upregulated genes in Cluster 4.

**PPI network construction.** The PPI networks of DEGs in Cluster 1 (Fig. 2A), 2 (Fig. 2B), 3 (Fig. 2C) and 4 (Fig. 2D) included 86, 18,875, 239 and 109 interactions, respectively. Based on connectivity degree, the hub genes with the highest degrees in the four clusters were: MDM2 oncogene, E3 ubiquitin protein ligase (*MDM2*), cyclin-dependent kinase 1 (*CDK1*), proteasome (prosome, macropain) 26S subunit, non-ATPase, 14 (*PSMD14*), translocator protein (18 kDa) (*TSPO*), respectively (Table I).

**GO and pathway enrichment analysis.** The present study performed GO and KEGG pathway analysis for DEGs in 4 clusters. The over-represented GO terms of DEGs in Cluster 1, 2, 3 and 4 were response to DNA damage stimulus, cell cycle, antigen processing and presentation of peptide antigen via MHC class I, and cell surface receptor signaling pathway, respectively (Table II). The significantly enriched KEGG pathways of cluster genes in Cluster 1, 2, 3 and 4 were the p53 signaling pathway, cell cycle, proteasome, and systemic lupus erythematosus, respectively (Table III).

**Functional annotation of DEGs in each cluster.** As presented in Table IV the present study revealed that with increased time 5 TFs and 13 TAGs in Cluster 1 were upregulated, 17 TFs and 49 TAGs in Cluster 2 were downregulated, 3 TFs and 3 TAGs in Cluster 3 were downregulated, 3 TFs and 15 TAGs in Cluster 4 were upregulated.

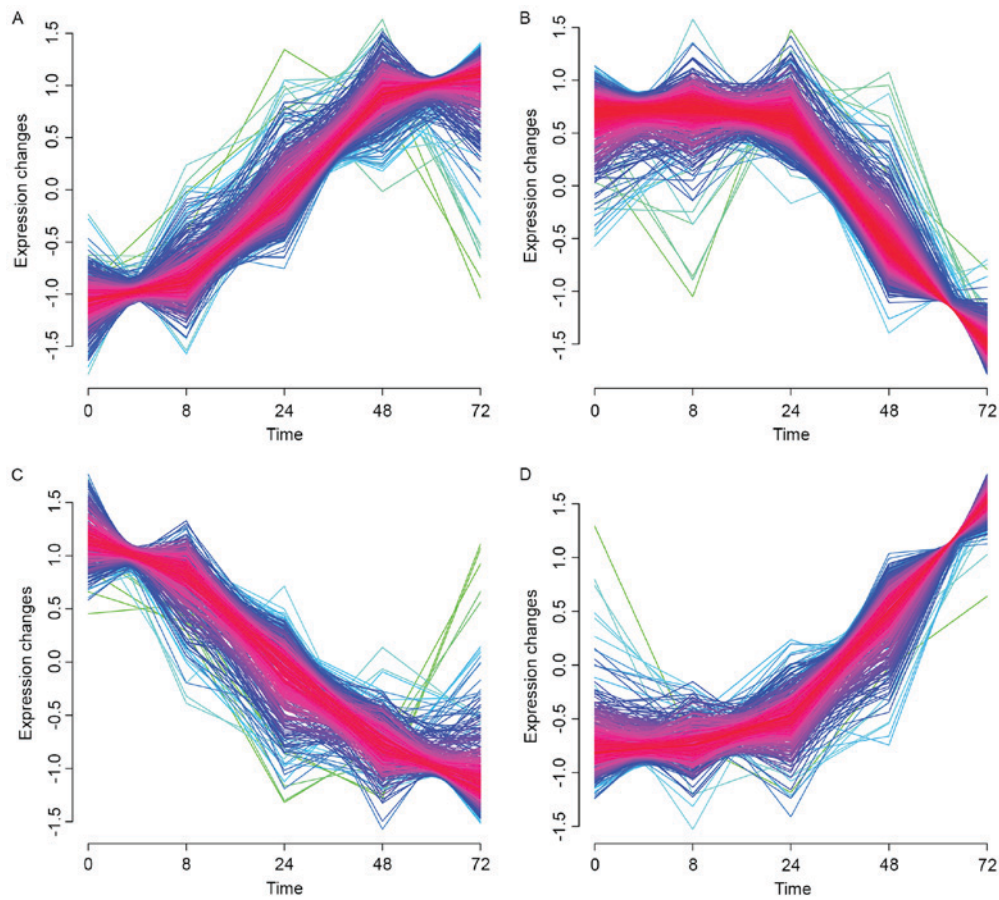


Figure 1. Clustering results of Cluster (A) 1, (B) 2, (C) 3 and (D) 4. Red shades indicate high membership values and green shades low membership values of genes.

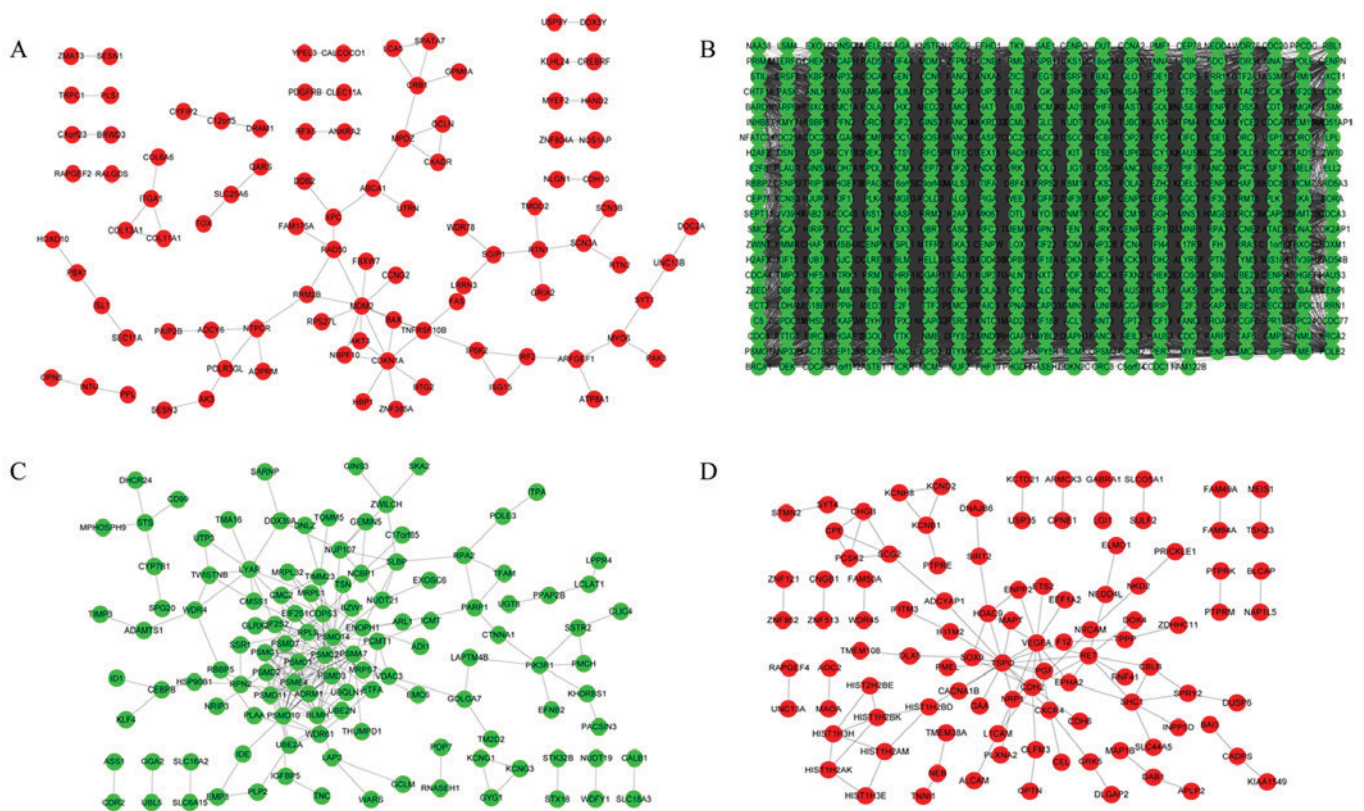


Figure 2. Protein-protein interaction networks of differentially expressed genes in Cluster (A) 1, (B) 2, (C) 3 and (D) 4. Red nodes indicate upregulated genes and green nodes indicate downregulated genes. The edges indicate interactions between these genes.



Table I. Top 10 nodes in the protein-protein interaction network.

A, Cluster 1	
Gene	Degree
MDM2	10
CDKN1A	8
TNFRSF10B	5
CRB1	4
MPDZ	4
NTPCR	4
RAD50	4
RTN1	4
ABCA1	3
ADCY6	3
B, Cluster 2	
Gene	Degree
CDK1	253
MAD2L1	251
RFC4	248
BUB1	241
NCAPG	239
CCNA2	238
CHEK1	236
CCNB1	233
NDC80	232
PBK	232
C, Cluster 3	
Gene	Degree
PSMD14	32
PSMC2	23
PSMD10	17
PSMD7	15
PSMC1	14
PSMD11	14
ADRM1	14
BLMH	13
PSMD3	13
PSMD1	13
D, Cluster 4	
Gene	Degree
TSPO	17
VEGFA	11
RET	9
CDH2	8
SHC1	6

Table I. Continued.

D, Cluster 4	
Gene	Degree
NRP1	6
CXCR4	5
HDAC9	5
SCG2	4
CHGB	4

## Discussion

The present study identified significant DEGs in a neuroblastoma cell line with CDK2 silencing, including *MDM2*, *CDK1*, *PSMD14* and *TSPO*. The genes with higher degrees in the PPI network were significantly enriched in the p53 signaling pathway, cell cycle and proteasome.

*MDM2* with the highest connectivity degrees in Cluster 1 was significantly upregulated in the neuroblastoma samples. The *MDM2* gene encodes a nuclear-localized E3 ubiquitin ligase, which is a critical effector of the *MYCN* oncogene in tumorigenesis and is a transcriptional target of *MYCN* in neuroblastoma (7,22). Elevated *MDM2* levels increase *MYCN*-induced genomic instability via regulating centrosome replication in the neuroblastoma (23). In addition, *MDM2* may bind to p53 at its transactivation domain with high affinity for negatively modulating its transcriptional activity and stability (24). A previous study favored the idea that the *MDM2*-p53 interaction was effectively involved in cellular processes via the p53 pathway (25). The p53 signaling pathway and its inactivation has a key regulatory role in neuroblastoma progression (26). Additionally, phosphorylation of MdmX by CDK2/Cdc2<sup>p34</sup> effectively regulates the nuclear export of *MDM2*, and thus has an important role in the regulation of p53 transcription and stability (27). Inhibition of p53-mediated apoptosis is a prerequisite for *MYC*-driven tumorigenesis in neuroblastoma (7). This may be the reason behind the upregulated expression of *MDM2* in neuroblastoma cells following CDK2 silencing. In the current study, *MDM2* was significantly enriched in the p53 signaling pathway. Therefore, the findings of the current study suggest that *MDM2* may function as an oncogene for promoting neuroblastoma progression via the p53 signaling pathway, and CDK2 may inhibit *MYC*-driven tumorigenesis in neuroblastoma by targeting *MDM2* and activating the p53 signaling pathway.

*PSMD14* is the hub gene in Cluster 3 with the higher degrees. This gene encodes a component of the 26S proteasome, which catalyzes the degradation of ubiquitinated intracellular proteins (28). The 26S proteasome may mediate the degradation of N-myc in neuroblastoma cells *in vivo* (29). Increased expression of the proteasome has an important role in the protective effects of sulforaphane against hydrogen peroxide-mediated cytotoxicity in neuroblastoma cells (30). Additionally, a *PSMD14* knockdown may restore sensitivity of Mcl1-dependent neuroblastoma to ABT-737 (a small molecule

Table II. The top 10 enriched GO-biological process terms of cluster genes.

A, Cluster 1			
ID	Description	Count	P-value
GO:0006974	Response to DNA damage stimulus	15	$2.71 \times 10^{-6}$
GO:0048699	Generation of neurons	18	$4.06 \times 10^{-5}$
GO:0009411	Response to UV	6	$2.49 \times 10^{-5}$
GO:0097202	Activation of cysteine-type endopeptidase activity	5	$1.39 \times 10^{-4}$
GO:0051050	Positive regulation of transport	11	$1.40 \times 10^{-4}$
GO:0032270	Positive regulation of cellular protein metabolic process	14	$2.35 \times 10^{-4}$
GO:0007267	Cell-cell signaling	16	$3.86 \times 10^{-4}$
GO:0045937	Positive regulation of phosphate metabolic process	11	$1.30 \times 10^{-3}$
GO:0050877	Neurological system process	16	$1.56 \times 10^{-3}$
GO:0051146	Striated muscle cell differentiation	6	$3.94 \times 10^{-3}$
B, Cluster 2			
ID	Description	Counts	P-value
GO:0007049	Cell cycle	231	0
GO:0006281	DNA repair	81	0
GO:0006260	DNA replication	72	0
GO:0006310	DNA recombination	51	0
GO:0000082	G1/S transition of mitotic cell cycle	41	0
GO:0000075	Cell cycle checkpoint	38	0
GO:0007051	Spindle organization	35	0
GO:0007126	Meiosis	32	0
GO:0007088	Regulation of mitosis	26	0
GO:0000086	G2/M transition of mitotic cell cycle	28	$2.22 \times 10^{-16}$
C, Cluster 3			
ID	Description	Count	P-value
GO:0002474	Antigen processing and presentation of peptide antigen via MHC class I	13	$3.21 \times 10^{-13}$
GO:0006521	Regulation of cellular amino acid metabolic process	11	$3.08 \times 10^{-13}$
GO:0006977	DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest	11	$1.38 \times 10^{-12}$
GO:0031145	Anaphase-promoting complex-dependent proteasomal ubiquitin-dependent protein catabolic process	11	$1.88 \times 10^{-11}$
GO:0000209	Protein polyubiquitination	14	$2.32 \times 10^{-11}$
GO:0016071	mRNA metabolic process	19	$1.07 \times 10^{-7}$
GO:0043248	Proteasome assembly	3	$2.04 \times 10^{-5}$
GO:0006406	mRNA export from nucleus	4	$1.35 \times 10^{-3}$
GO:0006369	Termination of RNA polymerase II transcription	3	$4.53 \times 10^{-3}$
GO:0006446	Regulation of translational initiation	3	$1.18 \times 10^{-2}$
D, Cluster 4			
ID	Description	Count	P-value
GO:0007166	Cell surface receptor signaling pathway	38	$1.79 \times 10^{-8}$
GO:0001525	Angiogenesis	12	$2.81 \times 10^{-6}$
GO:0045773	Positive regulation of axon extension	4	$8.88 \times 10^{-6}$
GO:0009968	Negative regulation of signal transduction	15	$3.97 \times 10^{-5}$

Table II. Continued.

D, Cluster 4			
ID	Description	Count	P-value
GO:0006334	Nucleosome assembly	6	5.21x10 <sup>-5</sup>
GO:0040013	Negative regulation of locomotion	6	4.04x10 <sup>-4</sup>
GO:0001657	Ureteric bud development	5	4.23x10 <sup>-4</sup>
GO:0042391	Regulation of membrane potential	8	5.87x10 <sup>-4</sup>
GO:0090090	Negative regulation of canonical Wnt receptor signaling pathway	4	1.60x10 <sup>-3</sup>
GO:0071495	Cellular response to endogenous stimulus	12	3.16x10 <sup>-3</sup>
GO, gene ontology.			

Table III. Enriched KEGG pathways of cluster genes.

A, Cluster 1			
ID	Description	Count	P-value
115	p53 signaling pathway	11	1.24x10 <sup>-12</sup>
5200	Pathways in cancer	7	9.90x10 <sup>-3</sup>
4510	Focal adhesion	6	3.48x10 <sup>-3</sup>
230	Purine metabolism	5	6.85x10 <sup>-3</sup>
5214	Glioma	4	1.33x10 <sup>-3</sup>
5218	Melanoma	4	1.85x10 <sup>-3</sup>
4210	Apoptosis	4	3.89x10 <sup>-3</sup>
5215	Prostate cancer	4	4.22x10 <sup>-3</sup>
5210	Colorectal cancer	3	1.09x10 <sup>-2</sup>
5220	Chronic myeloid leukemia	3	1.70x10 <sup>-2</sup>
4512	ECM-receptor interaction	3	2.53x10 <sup>-2</sup>
4012	ErbB signaling pathway	3	2.69x10 <sup>-2</sup>
240	Pyrimidine metabolism	3	3.74x10 <sup>-2</sup>
5219	Bladder cancer	2	3.91x10 <sup>-2</sup>
3420	Nucleotide excision repair	2	4.26x10 <sup>-2</sup>
B, Cluster 2			
ID	Description	Count	P-value
4110	Cell cycle	38	0
3030	DNA replication	23	0
4114	Oocyte meiosis	19	6.61x10 <sup>-10</sup>
230	Purine metabolism	16	2.72x10 <sup>-5</sup>
240	Pyrimidine metabolism	14	1.33x10 <sup>-6</sup>
3430	Mismatch repair	11	1.44x10 <sup>-11</sup>
3420	Nucleotide excision repair	11	4.70x10 <sup>-8</sup>
4914	Progesterone-mediated oocyte maturation	11	4.95x10 <sup>-5</sup>
3410	Base excision repair	10	2.58x10 <sup>-8</sup>
3440	Homologous recombination	9	7.45x10 <sup>-8</sup>
4115	p53 signaling pathway	9	1.87x10 <sup>-4</sup>
3018	RNA degradation	6	2.01x10 <sup>-2</sup>
310	Lysine degradation	5	1.01x10 <sup>-2</sup>
790	Folate biosynthesis	2	4.20x10 <sup>-2</sup>

Table III. Continued.

C, Cluster 3			
ID	Description	Count	P-value
3050	Proteasome	10	$7.56 \times 10^{-12}$
3013	RNA transport	6	$3.52 \times 10^{-3}$
3030	DNA replication	3	$5.19 \times 10^{-3}$
4141	Protein processing in endoplasmic reticulum	6	$5.44 \times 10^{-3}$
3410	Base excision repair	2	$4.17 \times 10^{-2}$
270	Cysteine and methionine metabolism	2	$4.88 \times 10^{-2}$
D, Cluster 4			
ID	Description	Count	P-value
5322	Systemic lupus erythematosus	7	$7.73 \times 10^{-5}$
4144	Endocytosis	6	$4.47 \times 10^{-3}$
4360	Axon guidance	5	$3.16 \times 10^{-3}$
4514	Cell adhesion molecules (CAMs)	5	$3.60 \times 10^{-3}$
5100	Bacterial invasion of epithelial cells	3	$1.71 \times 10^{-2}$
360	Phenylalanine metabolism	2	$7.58 \times 10^{-3}$
260	Glycine, serine and threonine metabolism	2	$2.57 \times 10^{-2}$
350	Tyrosine metabolism	2	$4.06 \times 10^{-2}$
5219	Bladder cancer	2	$4.24 \times 10^{-2}$

KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table IV. The functional annotation of cluster genes in PPI network.

Cluster no.	TF	TS genes	Oncogene	Other
Cluster 1	HAND2, RFX5, HOXD10, PBX1, ISL1	CDKN1A, ISG15, TNFRSF10B, HBP1, YPEL3, FBXW7, BTG2	IRF2, MDM2, AKT3, PDGFRB, PBX1	BAX
Cluster 2	HMGB2, BRCA1, BRIP1, EZH2, MYBL2, SSRP1, FOXM1, E2F7, HMGB1, BRCA2, TEAD1, TAF5, RBL1, GLI3, GTF2A1, MYBL1, LHX2	CHEK1, RBBP7, BRCA1, BARD1, BLM, FANCD2, BUB1B, CHEK2, FANCG, E2F1, LPL, GADD45G, BRCA2, RBL1, CDK2AP1, MLH1, ANP32A, LOX, RBM14, FH, CDKN2C	PTTG1, AURKA, CEP55, CCNA2, WHSC1, MYBL2, DEK, HMMR, CSE1L, RRAS, MYB, FGFR2, TEAD1, KIT, NTRK1, MYBL1	DNMT3B, BUB1, BIRC5, TACC3, PLK1, CCNE2, RAD54B, YEATS4, ANP32B, ANXA5, NIF3L1, LHX2
Cluster 3	TFAM, KLF4, ID1	TIMP3, IGFBP5		KLF4
Cluster 4	SOX9, MEIS1, ETS2	SPRY2, LGI1, BAI3, NRCAM, BLCAP, DUSP6, PTPRK, SIRT2, PRICKLE1	VEGFA, RET, MEIS1, ETS2	SHC1, CBLB

TF, transcription factor; TS, tumor suppressor.

inhibitor of Bcl2, BclXL and BclW), thus decreasing the activity of Bcl2, BclXL and BclW (31). Bcl2 family proteins have important roles in neutralizing activated BCL2 like 11 and evading apoptosis in neuroblastoma cells (32,33). Therefore, the findings of the current study suggest that *PSMD14* may contribute to neuroblastoma progression via the proteasome. It

is of note that the clustering analysis performed in the current study revealed that the expression pattern of genes in Cluster 3 at different time points was evidently opposite to the one observed in Cluster 1. Therefore, it is possible that a synthetic suppression effect occurred between these genes in Cluster 3 and Cluster 1 to some extent. Liang *et al* (34) demonstrated

that downregulation of *PSMD14* was involved in the activation of p53-regulated pro-apoptotic signaling pathways and the activity of p53 was associated with MDM2 expression (34). Additionally, p53 regulates the expression of cyclin dependent kinase inhibitor 1A, which mediates the p53-dependent cell cycle arrest at the G1 phase via binding and thus inhibiting the activity of CDK2. Therefore, the findings of the present study also suggest that CDK2 may have a key role in neuroblastoma progression by regulating the expression of p53, which may be due to the synthetically lethal relationship between *MDM2* and *PSMD14*.

CDK1 has an important role in cell cycle regulation by governing the transition from G2 to M phase and cell cycle regulation is important for cell proliferation (35,36). The CDK1 inhibitors induce G2 arrest in various cell types and effectively downregulate the expression of *MYCN*, which in turn reduce the transcriptional activation of *MYCN* on the survivin promoter in neuroblastoma cells (37). In the present study, CDK1 was significantly involved in cell cycle. Therefore, CDK1 may be involved in neuroblastoma progression through the cell cycle. However, previous studies have confirmed that CDK1 alone is sufficient to drive the mammalian cell cycle and the genetic ablation of CDK2 may be compensated for by CDK1 (38,39). A previous study determined that despite CDK2 inhibition, the proliferation of cancer cells was due to the expression of CDK1 to some extent (39). In the current study, the expression of CDK1 was downregulated following CDK2 silencing; therefore, it is possible for CDK2 to contribute to neuroblastoma progression via regulation of CDK1 expression.

TSPO is a transmembrane protein associated with the mitochondrial permeability pore, mitochondrial transport has an important role in the initiation of the apoptotic cascade (40). A previous study revealed that TSPO ligands are capable of inducing apoptosis in various types of cancers, such as hepatocellular carcinoma, colorectal cancer, esophageal cancer and glioma (41). TSPO ligand PK11195 induces apoptosis and leads to cell cycle arrest in neuroblastoma cell lines at micromolar concentrations (42). Therefore, TSPO may induce apoptosis in neuroblastoma cells and is involved in cell cycle. However, with CDK2 silencing, the expression of TSPO has been observed to be upregulated. Therefore, CDK2 may promote neuroblastoma progression by reducing TSPO expression. Due to the effect of synthetic suppression observed between TSPO and CDK1 in the present study, it is possible that CDK2 may be involved in neuroblastoma progression via regulation of the interaction of TSPO and CDK1 in the cell cycle.

However, the relatively small sample size is a limitation of the current study. In addition, there is no experimental evaluation of the present study. Additional experiments, such as expression validation or knockdown assay are required to confirm the current observations.

In conclusion, *MDM2*, *CDK1*, *PSMD14* and *TSPO* may be key target genes of CDK2, and CDK2 may play an important role in neuroblastoma progression by targeting these genes. *MDM2* may function as an oncogene that promotes neuroblastoma tumorigenesis via the p53 signaling pathway. *PSMD14* may allow neuroblastoma cells to evade apoptosis in via proteasome. *TSPO* and *CDK1* may be involved in neuroblastoma progression by regulating the cell cycle. CDK2 may promote

neuroblastoma progression by regulating the expression of *MDM2*, *PSMD14*, *CDK1* and *TSPO*.

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