Bioinformatics analysis of the CDK2 functions in neuroblastoma

LIJUAN BO[1*], BO WEI[2*], ZHANFENG WANG[2], DALIANG KONG[3], ZHENG GAO[2] and ZHUANG MIAO[2]

Departments of [1]Infections, [2]Neurosurgery and [3]Orthopaedics, China-Japan Union Hospital of Jilin University, Changchun, Jilin 130033, P.R. China

Received December 20, 2016; Accepted November 14, 2017

DOI: 10.3892/mmr.2017.8368

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

Correspondence to: Dr Zhuang Miao, Department of Neurosurgery, China-Japan Union Hospital of Jilin University, 126 Xiantai Street, Changchun, Jilin 130033, P.R. China
E-mail: miaozhuang99@163.com

*Contributed equally

Key words: neuroblastoma, cyclin-dependent kinase 2, differentially expressed genes, pathways analysis, protein-protein interaction

(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.

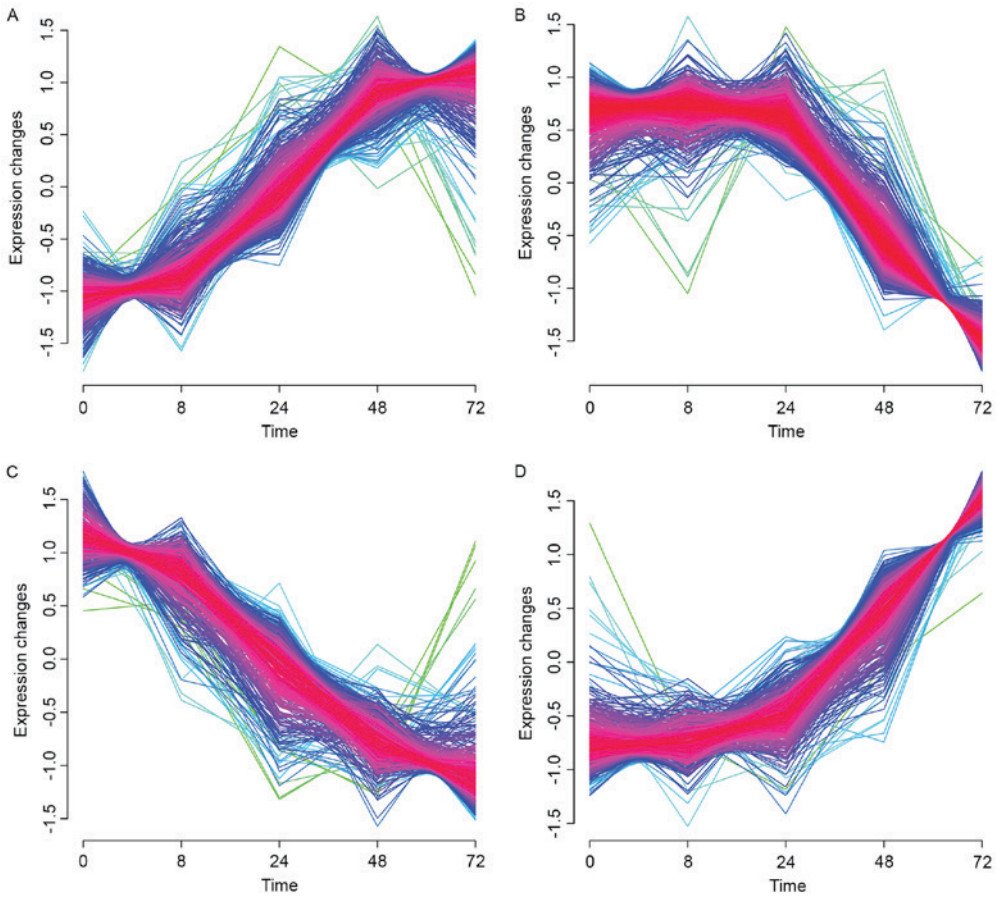

Figure 1. Clustering results of Cluster (A) 1, (B) 2, (C) 3 and (D) 4. Red shades indicate high membership values and green shades low membership values of genes.

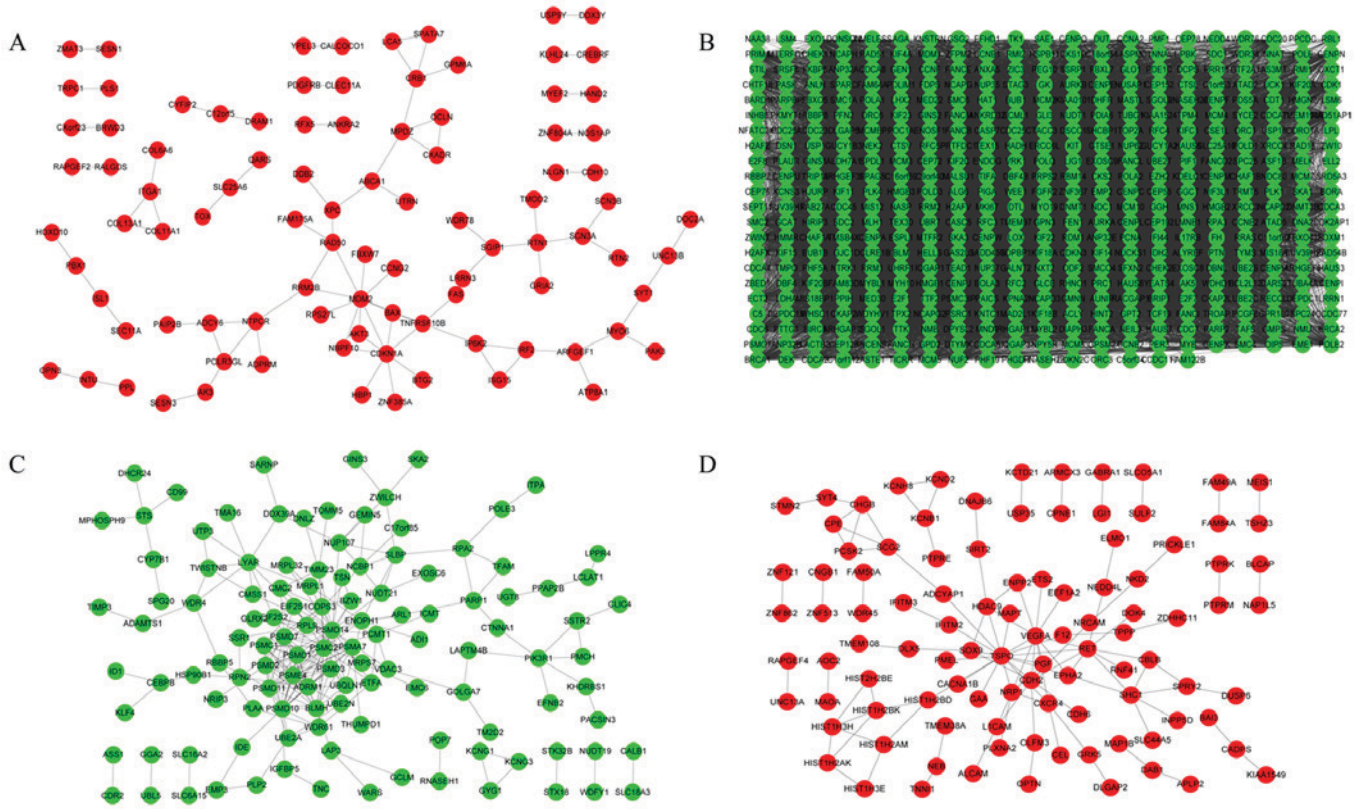

Figure 2. Protein-protein interaction networks of differentially expressed genes in Cluster (A) 1, (B) 2, (C) 3 and (D) 4. Red nodes indicate upregulated genes and green nodes indicate downregulated genes. The edges indicate interactions between these genes.

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^{p34} 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	2.71×10^{-6}
GO:0048699	Generation of neurons	18	4.06×10^{-5}
GO:0009411	Response to UV	6	2.49×10^{-5}
GO:0097202	Activation of cysteine-type endopeptidase activity	5	1.39×10^{-4}
GO:0051050	Positive regulation of transport	11	1.40×10^{-4}
GO:0032270	Positive regulation of cellular protein metabolic process	14	2.35×10^{-4}
GO:0007267	Cell-cell signaling	16	3.86×10^{-4}
GO:0045937	Positive regulation of phosphate metabolic process	11	1.30×10^{-3}
GO:0050877	Neurological system process	16	1.56×10^{-3}
GO:0051146	Striated muscle cell differentiation	6	3.94×10^{-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	2.22×10^{-16}

C, Cluster 3

ID	Description	Count	P-value
GO:0002474	Antigen processing and presentation of peptide antigen via MHC class I	13	3.21×10^{-13}
GO:0006521	Regulation of cellular amino acid metabolic process	11	3.08×10^{-13}
GO:0006977	DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest	11	1.38×10^{-12}
GO:0031145	Anaphase-promoting complex-dependent proteasomal ubiquitin-dependent protein catabolic process	11	1.88×10^{-11}
GO:0000209	Protein polyubiquitination	14	2.32×10^{-11}
GO:0016071	mRNA metabolic process	19	1.07×10^{-7}
GO:0043248	Proteasome assembly	3	2.04×10^{-5}
GO:0006406	mRNA export from nucleus	4	1.35×10^{-3}
GO:0006369	Termination of RNA polymerase II transcription	3	4.53×10^{-3}
GO:0006446	Regulation of translational initiation	3	1.18×10^{-2}

D, Cluster 4

ID	Description	Count	P-value
GO:0007166	Cell surface receptor signaling pathway	38	1.79×10^{-8}
GO:0001525	Angiogenesis	12	2.81×10^{-6}
GO:0045773	Positive regulation of axon extension	4	8.88×10^{-6}
GO:0009968	Negative regulation of signal transduction	15	3.97×10^{-5}

Table II. Continued.

D, Cluster 4

ID	Description	Count	P-value
GO:0006334	Nucleosome assembly	6	5.21×10^{-5}
GO:0040013	Negative regulation of locomotion	6	4.04×10^{-4}
GO:0001657	Ureteric bud development	5	4.23×10^{-4}
GO:0042391	Regulation of membrane potential	8	5.87×10^{-4}
GO:0090090	Negative regulation of canonical Wnt receptor signaling pathway	4	1.60×10^{-3}
GO:0071495	Cellular response to endogenous stimulus	12	3.16×10^{-3}

GO, gene ontology.

Table III. Enriched KEGG pathways of cluster genes.

A, Cluster 1

ID	Description	Count	P-value
115	p53 signaling pathway	11	1.24×10^{-12}
5200	Pathways in cancer	7	9.90×10^{-3}
4510	Focal adhesion	6	3.48×10^{-3}
230	Purine metabolism	5	6.85×10^{-3}
5214	Glioma	4	1.33×10^{-3}
5218	Melanoma	4	1.85×10^{-3}
4210	Apoptosis	4	3.89×10^{-3}
5215	Prostate cancer	4	4.22×10^{-3}
5210	Colorectal cancer	3	1.09×10^{-2}
5220	Chronic myeloid leukemia	3	1.70×10^{-2}
4512	ECM-receptor interaction	3	2.53×10^{-2}
4012	ErbB signaling pathway	3	2.69×10^{-2}
240	Pyrimidine metabolism	3	3.74×10^{-2}
5219	Bladder cancer	2	3.91×10^{-2}
3420	Nucleotide excision repair	2	4.26×10^{-2}

B, Cluster 2

ID	Description	Count	P-value
4110	Cell cycle	38	0
3030	DNA replication	23	0
4114	Oocyte meiosis	19	6.61×10^{-10}
230	Purine metabolism	16	2.72×10^{-5}
240	Pyrimidine metabolism	14	1.33×10^{-6}
3430	Mismatch repair	11	1.44×10^{-11}
3420	Nucleotide excision repair	11	4.70×10^{-8}
4914	Progesterone-mediated oocyte maturation	11	4.95×10^{-5}
3410	Base excision repair	10	2.58×10^{-8}
3440	Homologous recombination	9	7.45×10^{-8}
4115	p53 signaling pathway	9	1.87×10^{-4}
3018	RNA degradation	6	2.01×10^{-2}
310	Lysine degradation	5	1.01×10^{-2}
790	Folate biosynthesis	2	4.20×10^{-2}

Table III. Continued.

C, Cluster 3

ID	Description	Count	P-value
3050	Proteasome	10	$7.56x10^{-12}$
3013	RNA transport	6	$3.52x10^{-3}$
3030	DNA replication	3	$5.19x10^{-3}$
4141	Protein processing in endoplasmic reticulum	6	$5.44x10^{-3}$
3410	Base excision repair	2	$4.17x10^{-2}$
270	Cysteine and methionine metabolism	2	$4.88x10^{-2}$

D, Cluster 4

ID	Description	Count	P-value
5322	Systemic lupus erythematosus	7	$7.73x10^{-5}$
4144	Endocytosis	6	$4.47x10^{-3}$
4360	Axon guidance	5	$3.16x10^{-3}$
4514	Cell adhesion molecules (CAMs)	5	$3.60x10^{-3}$
5100	Bacterial invasion of epithelial cells	3	$1.71x10^{-2}$
360	Phenylalanine metabolism	2	$7.58x10^{-3}$
260	Glycine, serine and threonine metabolism	2	$2.57x10^{-2}$
350	Tyrosine metabolism	2	$4.06x10^{-2}$
5219	Bladder cancer	2	$4.24x10^{-2}$

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*.

Acknowledgements

The present study was supported by The Natural Science Foundation of Jilin Province (grant nos. 20150204086SF and 20150101160JC) and The High Technology Research and Development Program of Jilin Province of China (grant nos: 2015Y032-4 and 2014G074).

References

1. Reck M, Popat S, Reinmuth N, De Ruysscher D, Kerr KM and Peters S; ESMO Guidelines Working Group: Metastatic non-small-cell lung cancer (NSCLC): ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 3 (Suppl 25): iii27-iii39, 2014.
2. George RE, Sanda T, Hanna M, Fröhling S, Luther W II, Zhang J, Ahn Y, Zhou W, London WB, McGrady P, *et al*: Activating mutations in ALK provide a therapeutic target in neuroblastoma. Nature 455: 975-978, 2008.
3. Mossé YP, Laudenslager M, Longo L, Cole KA, Wood A, Attiyeh EF, Laquaglia MJ, Sennett R, Lynch JE, Perri P, *et al*: Identification of ALK as a major familial neuroblastoma predisposition gene. Nature 455: 930-935, 2008.
4. George RE, Li S, Medeiros-Nancarrow C, Neuberg D, Marcus K, Shamberger RC, Pulsipher M, Grupp SA and Diller L: High-risk neuroblastoma treated with tandem autologous peripheral-blood stem cell-supported transplantation: Long-term survival update. J Clin Oncol 24: 2891-2896, 2006.
5. Schwab M, Varmus HE, Bishop JM, Grzeschik KH, Naylor SL, Sakaguchi AY, Brodeur G and Trent J: Chromosome localization in normal human cells and neuroblastomas of a gene related to c-myc. Nature 308: 288-291, 1984.
6. Cole MD and Cowling VH: Transcription-independent functions of MYC: Regulation of translation and DNA replication. Nat Rev Mol Cell Biol 9: 810-815, 2008.
7. Slack A, Chen Z, Tonelli R, Pule M, Hunt L, Pession A and Shohet JM: The p53 regulatory gene MDM2 is a direct transcriptional target of MYCN in neuroblastoma. Proc Natl Acad Sci USA 102: 731-736, 2005.
8. Meyer N, Kim SS and Penn LZ: The Oscar-worthy role of Myc in apoptosis. Semin Cancer Biol 16: 275-287, 2006.
9. Molenaar JJ, Ebus ME, Geerts D, Koster J, Lamers F, Valentijn LJ, Westerhout EM, Versteeg R and Caron HN: Inactivation of CDK2 is synthetically lethal to MYCN over-expressing cancer cells. Proc Natl Acad Sci USA 106: 12968-12973, 2009.
10. Vermeulen K, Van Bockstaele DR and Berneman ZN: The cell cycle: A review of regulation, deregulation and therapeutic targets in cancer. Cell Prolif 36: 131-149, 2003.
11. Malumbres M and Barbacid M: Mammalian cyclin-dependent kinases. Trends Biochem Sci 30: 630-641, 2005.
12. Shapiro GI: Cyclin-dependent kinase pathways as targets for cancer treatment. J Clin Oncol 24: 1770-1783, 2006.
13. Hall C, Nelson DM, Ye X, Baker K, DeCaprio JA, Seeholzer S, Lipinski M and Adams PD: HIRA, the human homologue of yeast Hir1p and Hir2p, is a novel cyclin-cdk2 substrate whose expression blocks S-phase progression. Mol Cell Biol 21: 1854-1865, 2001.
14. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U and Speed TP: Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 4: 249-264, 2003.
15. Futschik ME and Carlisle B: Noise-robust soft clustering of gene expression time-course data. J Bioinform Comput Biol 3: 965-988, 2005.
16. Kumar L and E Futschik M: Mfuzz: A software package for soft clustering of microarray data. Bioinformation 2: 5-7, 2007.
17. von Mering C, Huynen M, Jaeggi D, Schmidt S, Bork P and Snel B: STRING: A database of predicted functional associations between proteins. Nucleic Acids Res 31: 258-261, 2003.
18. 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 25: 25-29, 2000.

19. Kanehisa M and Goto S: KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28: 27-30, 2000.
20. Zhao M, Sun J and Zhao Z: TSGene: A web resource for tumor suppressor genes. Nucleic Acids Res 41 (Database Issue): D970-D976, 2013.
21. Chen JS, Hung WS, Chan HH, Tsai SJ and Sun HS: *In silico* identification of oncogenic potential of fyn-related kinase in hepatocellular carcinoma. Bioinformatics 29: 420-427, 2013.
22. Slack A and Shohet JM: MDM2 as a critical effector of the MYCN oncogene in tumorigenesis. Cell Cycle 4: 857-860, 2005.
23. Slack AD, Chen Z, Ludwig AD, Hicks J and Shohet JM: MYCN-directed centrosome amplification requires MDM2-mediated suppression of p53 activity in neuroblastoma cells. Cancer Res 67: 2448-2455, 2007.
24. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, *et al*: In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science 303: 844-848, 2004.
25. Meek DW and Knippschild U: Posttranslational modification of MDM2. Mol Cancer Res 1: 1017-1026, 2003.
26. Tweddle DA, Pearson AD, Haber M, Norris MD, Xue C, Flemming C and Lunec J: The p53 pathway and its inactivation in neuroblastoma. Cancer Lett 197: 93-98, 2003.
27. Elias B, Laine A and Ronai Z: Phosphorylation of MdmX by CDK2/Cdc2(p34) is required for nuclear export of Mdm2. Oncogene 24: 2574-2579, 2005.
28. Tanaka K: The proteasome: Overview of structure and functions. Proc Jpn Acad Ser B Phys Biol Sci 85: 12-36, 2009.
29. Bonvini P, Nguyen P, Trepel J and Neckers LM: In vivo degradation of N-myc in neuroblastoma cells is mediated by the 26S proteasome. Oncogene 16: 1131-1139, 1998.
30. Kwak MK, Cho JM, Huang B, Shin S and Kensler TW: Role of increased expression of the proteasome in the protective effects of sulforaphane against hydrogen peroxide-mediated cytotoxicity in murine neuroblastoma cells. Free Radic Biol Med 43: 809-817, 2007.
31. Laetsch T, Liu X, Vu A, Sliozberg M, Vido M, Elci OU, Goldsmith KC and Hogarty MD: Multiple components of the spliceosome regulate Mcl1 activity in neuroblastoma. Cell Death Dis 5: e1072, 2014.
32. Goldsmith KC, Lestini BJ, Gross M, Ip L, Bhumbla A, Zhang X, Zhao H, Liu X and Hogarty MD: BH3 response profiles from neuroblastoma mitochondria predict activity of small molecule Bcl-2 family antagonists. Cell Death Differ 17: 872-882, 2010.
33. Goldsmith KC, Gross M, Peirce S, Luyindula D, Liu X, Vu A, Sliozberg M, Guo R, Zhao H, Reynolds CP and Hogarty MD: Mitochondrial Bcl-2 family dynamics define therapy response and resistance in neuroblastoma. Cancer Res 72: 2565-2577, 2012.
34. Liang J, Parchaliuk D, Medina S, Sorensen G, Landry L, Huang S, Wang M, Kong Q and Booth SA: Activation of p53-regulated pro-apoptotic signaling pathways in PrP-mediated myopathy. BMC Genomics 10: 201, 2009.
35. Goga A, Yang D, Tward AD, Morgan DO and Bishop JM: Inhibition of CDK1 as a potential therapy for tumors over-expressing MYC. Nat Med 13: 820-827, 2007.
36. Bettayeb K, Oumata N, Echalier A, Ferandin Y, Endicott JA, Galons H and Meijer L: CR8, a potent and selective, roscovitine-derived inhibitor of cyclin-dependent kinases. Oncogene 27: 5797-5807, 2008.
37. Chen Y, Tsai YH and Tseng SH: Inhibition of cyclin-dependent kinase 1-induced cell death in neuroblastoma cells through the microRNA-34a-MYCN-survivin pathway. Surgery 153: 4-16, 2013.
38. Santamaría D, Barrière C, Cerqueira A, Hunt S, Tardy C, Newton K, Cáceres JF, Dubus P, Malumbres M and Barbacid M: Cdk1 is sufficient to drive the mammalian cell cycle. Nature 448: 811-815, 2007.
39. Tetsu O and McCormick F: Proliferation of cancer cells despite CDK2 inhibition. Cancer Cell 3: 233-245, 2003.
40. Casellas P, Galiegue S and Basile AS: Peripheral benzodiazepine receptors and mitochondrial function. Neurochem Int 40: 475-486, 2002.
41. Santidrián AF, Cosialls AM, Coll-Mulet L, Iglesias-Serret D, de Frias M, González-Gironès DM, Campàs C, Domingo A, Pons G and Gil J: The potential anticancer agent PK11195 induces apoptosis irrespective of p53 and ATM status in chronic lymphocytic leukemia cells. Haematologica 92: 1631-1638, 2007.
42. Mendonça-Torres MC and Roberts SS: The translocator protein (TSPO) ligand PK11195 induces apoptosis and cell cycle arrest and sensitizes to chemotherapy treatment in pre- and post-relapse neuroblastoma cell lines. Cancer Biol Ther 14: 319-326, 2013.