Microarray data analysis of neuroblastoma: Expression of SOX2 downregulates the expression of MYCN

JUNTAO BAO¹, LUYING QIN², LINGLING CUI³, XIAOHUI WANG¹, QINGLEI MENG¹, LINCHAO ZHU¹ and SHUFENG ZHANG¹

¹Department of Pediatric Surgery, Henan Provincial People's Hospital, Zhengzhou, Henan 450003; ²Nursing College, Zhengzhou University; ³College of Public Health, Zhengzhou University, Zhengzhou, Henan 450001, P.R. China

Received November 17, 2014; Accepted August 4, 2015

DOI: 10.3892/mmr.2015.4311

Abstract. The present study aimed to identify the genes directly or indirectly correlated with the amplification of MYCN in neuroblastoma (NB). Microarray data (GSE53371) were downloaded from Gene Expression Omnibus, and included 10 NB cell lines with MYCN amplification and 10 NB cell lines with normal MYCN copy numbers. Differentially expressed genes (DEGs) were identified using the Linear Models for Microarray Data package, and a false discovery rate of <0.05 and llog₂FC (fold change)|>1 were selected as cut-off criteria. Hierarchical clustering analysis and Gene Ontology analysis were respectively performed for the DEGs using the Pheatmap package in R language and The Database for Annotation, Visualization and Integrated Discovery. A protein-protein interaction network (PPI) was constructed for the DEGs using the Search Tool for the Retrieval of Interacting Genes database. Pathway analysis was performed for the DEGs in the PPI network using the WEB-based GEne SeT AnaLysis Toolkit. The correlation between MYCN and the key gene associated with MYCN was determined using Pearson's correlation coefficient. In total, 137 downregulated and 35 upregulated DEGs were identified. Functional enrichment analysis indicated that KCNMB4 was involved in the regulation of action potential in neuron term, and the FOS, GLI3 and GLI1 genes were involved in the extracellular matrix-receptor interaction pathway. The PPI network and correlation analysis revealed that the expression of SOX2 was directly correlated with the expression of MYCN, and the correlation coefficient of SOX2 and MYCN

Correspondence to: Dr Shufeng Zhang, Department of Pediatric Surgery, Henan Provincial People's Hospital, 7 Weft Five Road, Zhengzhou, Henan 450003, P.R. China E-mail: shufengzhangzhsh@163.com

Dr Luying Qin, Nursing College, Zhengzhou University, 100 Science Avenue, Zhengzhou, Henan 450001, P.R. China E-mail: qinluyingemail@163.com

Key words: neuroblastoma, hierarchical clustering, functional enrichment, correlation analysis

was -0.83. Therefore, *SOX2*, *KCNMB4*, *FOS*, *GLI3* and *GLI1* may be involved in the pathogenesis of NB, with the expression of *SOX2* downregulating the expression of *MYCN*.

Introduction

Neuroblastoma (NB) is a cancer of the peripheral sympathetic neurons, which occurs during childhood, and the percentage of NB of all pediatric oncology-associated mortality is ~10% worldwide (1). NB predominately occurs in young children, of which the average age is 17 months (2) NB derives from undifferentiated neural crest cells and metastasizes to other organs (3). Patients with NB have poor prognosis with a 5-year survival rate of no more than 30% (4).

With the development of the field of biology, increasing numbers of markers correlated with NB have been identified. MYCN is a well-known prognostic marker of NB (5), and MYCN amplification is the most significant molecular marker of risk in NB (6). Tang et al demonstrated that patients with NB exhibiting low expression levels of MYCN and TrkA have a 5-year survival rate of 63.7%, whereas the 5-year survival rate of patients with high expression levels of the two is 88.1% (7). In addition, the alternative TrkAIII splice variant is involved in the pathogenesis of advanced stage human NB through microtubules, which are involved in the promotion and maintenance of NB cells (8). In addition to these reports, NCYM has been observed to act as a cis-antisense gene of MYCN, and contributes to the stabilization of MYCN in human NB by inhibiting $GSK3\beta$ (9). NCYM, co-amplified with MYCN, is involved in the pathogenesis of NB (10). Although several studies have reported that MYCN is key in the pathogenesis of NB, the genes correlated with MYCN amplification remain to be fully elucidated. Therefore, the present study aimed to examine the molecular mechanism of MYCN amplification in NB.

Wu *et al* analyzed expression profiles using Ingenuity Pathway Analysis, and found that the aryl hydrocarbon receptor downregulates the expression of MYCN through the mediation of E2F1 in the protein-protein interaction (PPI) network, and further confirmed that the aryl hydrocarbon receptor regulates the activity of the MYCN promoter and results in the downregulated expression of MYCN (11). To identify more genes correlated with MYCN in the present study, the determination of differentially expressed genes (DEGs) between NB cell lines exhibiting *MYCN* amplification (*MYCN* amplification group) and NB cell lines with a normal *MYCN* copy number (control group) were identified. Hierarchical clustering and Gene Ontology (GO) analysis were performed for these DEGs, and a protein-protein interaction (PPI) network was constructed for the DEGs to identify the key genes associated with *MYCN*. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was also performed for the DEGs in PPI network. The correlation of *MYCN* and the key gene associated with to *MYCN* was determined using Pearson's correlation coefficient, in order to investigate whether their association was synergistic or antagonistic.

Materials and methods

Microarray data. Microarray data (accession. no. GSE53371) were downloaded from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) (11). The microarray platform of GSE53371 was GPL887 Agilent-012097 Human 1A Microarray (V2) G4110B (Feature Number version; Agilent Technologies, Inc., Santa Clara, CA, USA). A total of 20 samples were available, which included 10 NB cell lines with *MYCN* amplification and 10 NB cell lines with a normal *MYCN* copy number.

Data preprocessing and identification of DEGs. Normalization of the microarray data were performed using a median normalization method (12). The DEGs were identified using the Linear Models for Microarray Data (Limma) package (13). The raw P-value was adjusted into a false discovery rate (FDR) using the Benjamini & Hochberg method (14,15). The genes with an FDR <0.05 and $llog_2FC$ (fold change)|>1 were considered significantly different between the *MYCN* amplification group and the control group.

Hierarchical clustering analysis of DEGs. Based on the Euclidean distance, which is the actual distance between two points that may be calculated using the Pythagorean formula, hierarchical clustering analysis (16) was performed to evaluate the sample specificity. In the hierarchical clustering analysis, the expression value of the DEGs were extracted, and subsequent analysis was performed of the DEGs using the Pheatmap package in R language (http://cran.r-project. org/web/packages/pheatmap/index.html) (16,17).

Functional enrichment analysis of DEGs. The Database for Annotation, Visualization and Integrated Discovery (DAVID) is a comprehensive functional annotation tool (18). Gene Ontology (GO) analysis was performed for the DEGs using the DAVID database (http://david.abcc.ncifcrf.gov/). The raw P-value was adjusted into the FDR using Benjamini & Hochberg's method and an FDR <0.05 was selected as the cut-off criterion.

Construction of the PPI network. The Search Tool for the Retrieval of Interacting Genes (STRING) database is used to collect and predict PPI associations (19). In the present

study, a PPI network was constructed for the DEGs using STRING, and a confidence score >0.4 was selected as the cut-off criterion. Subsequent visualization of the PPI network was performed using Cytoscape (http://cytoscape.org/) (20).

Pathway analysis of DEGs in the PPI network. The WEB-based GEne SeT AnaLysis Toolkit (WebGestalt) aims to examine large sets of genes, and is used for functional enrichment analysis, including GO enrichment, pathway enrichment and transcription factor analysis, by integrating functional categories (21,22). KEGG (http://www.genome. ad.jp/kegg) pathway enrichment analysis was performed for the DEGs in the PPI network using WebGestalt, and P<0.05 was selected as the cut-off criterion.

Correlation analysis. Based on the PPI network, the expression values of the genes correlated directly with *MYCN* were extracted, and correlation analysis was performed using the expression values of the genes. For this correlation analysis, the correlation of *MYCN* and the key gene associated with *MYCN* was determined using Pearson's correlation coefficient (23) with SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Identification of DEGs. In total, 172 DEGs between the *MYCN* amplification group and the control group were identified, which included 137 downregulated DEGs and 35 upregulated DEGs.

Hierarchical clustering analysis of the DEGs. Following hierarchical clustering analysis, there were two distinct gene clusters identified, including the *MYCN* amplification cluster and control cluster (Fig. 1). These results suggested that there were two different gene expression patterns with marked color differences, which were available for use to distinguish between the *MYCN* amplification samples and the control samples.

GO enrichment analysis of the DEGs. GO enrichment analysis revealed that the five enriched terms were as follows: Regulation of action potential in neuron (P=1.24E-05), myelination (P=2.95E-05), regulation of action potential (P=4.71E-05), ensheathment of neurons (P=4.85E-05) and axon ensheathment (P=4.85E-05), as listed in Table I. The KCNMB4, PLP1, CLDN1, LGI4, MAL, PMP22 and GAL3ST1 genes were involved in the regulation of action potential in neuron biological process, and the percentage of the genes enriched in this term was 21.88% (Fig. 2).

PPI network analysis of DEGs and pathway analysis. Analysis of the PPI network revealed that there were 85 nodes and 142 gene links, and *SOX2* was identified as the key gene correlated directly with *MYCN* (Fig. 3).

KEGG pathway enrichment analysis of the DEGs in the PPI network indicated that the *ASPA*, *MAOA* and *ALDH1A3* genes were involved in the histidine metabolism pathway (P=0.012376), and the *LAMA2*, *FOS*, *ITGA6*, *GLI3*, *GLI1* and *RAD51* genes were involved in the extracellular matrix (ECM)-receptor interaction pathway (P=0.040716; Table II).

GO ID	GO term	Gene count	P-value	FDR	Gene
GO:0019228	Regulation of action potential in neuron	7	1.24E-05	0.015898	KCNMB4, PLP1, CLDN1, LGI4, MAL, PMP22, GAL3ST1
GO:0042552	Myelination	6	2.95E-05	0.018878	PLP1, CLDN1, LGI4, MAL, PMP22, GAL3ST1
GO:0001508	Regulation of action potential	7	4.71E-05	0.020080	KCNMB4, PLP1, CLDN1, LGI4, MAL, PMP22, GAL3ST1
GO:0007272	Ensheathment of neurons	6	4.85E-05	0.015539	PLP1, CLDN1, LGI4, MAL, PMP22, GAL3ST1
GO:0008366	Axon ensheathment	6	4.85E-05	0.015539	PLP1, CLDN1, LGI4, MAL, PMP22, GAL3ST1
GO, Gene Ontol	ogy; FDR, false discovery rate.				

Table I. Top five most enriched GO terms for the differentially expressed genes.

Table II. Top two most enriched KEGG pathways of the differentially expressed genes in the protein-protein interaction network.

ID	KEGG pathway	Gene count	P-value	Gene
hsa00340	Histidine metabolism	3	0.012376	ASPA, MAOA, ALDH1A3
hsa04512	ECM-receptor interaction	6	0.040716	LAMA2, FOS, ITGA6, GLI3, GLI1, RAD51

KEGG, Kyoto Encyclopedia of Genes and Genomes; ECM, extracellular matrix.



Figure 1. Hierarchical clustering analysis of differentially expressed genes. Red represents the higher levels of gene expression; blue represents lower levels of gene expression.

Correlation analysis of genes. Correlation analysis indicated that the expression of *SOX2* was inversely correlated with the expression of *MYCN* in the NB cell lines, and the correlation coefficient of *SOX2* and *MYCN* was -0.83 (Fig. 4).

Discussion

In the present study, 172 DEGs between the *MYCN* amplification group and control group were identified, which included 137 downregulated and 35 upregulated DEGs. Functional enrichment analysis indicated that the *KCNMB4* gene was involved in the regulation of action potential in neuron pethway, and the *FOS*, *GLI3* and *GLI1* genes were involved in the ECM-receptor interaction pathway. Correlation analysis demonstrated that the expression of *SOX2* was inversely correlated with the expression of *MYCN* in the NB cell lines.

Neurons encode and convey information by generating action potentials (24). According to a previous report by Hall *et al*, the expression of specific Kv3.1 glycoprotein, which is a voltage-gated potassium (Kv) channel, has an



Figure 2. Pie chart of Gene Ontology enrichment analysis for the differentially expressed genes. The percentage was proportional to the differentially expressed genes enriched in each function.



Figure 3. Protein-protein interaction network of differentially expressed genes. The blue square node represents *MYCN*; the green square node represents *SOX2*. The line between two genes represents their interaction.

impact on the wave form of action potentials and *N*-glycans associated with the Kv3.1 protein affect the migratory rate of The NB cell (25). In addition, Leung *et al* demonstrated that the voltage-gated Kv channel has an effect on cAMP-stimulated neuritogenesis in mouse NB N2A cells (26). These findings suggest that the voltage-gated Kv channels are important in the development of NB through the modulation of action potentials. Large-conductance Ca²⁺-activated K⁺ (BK) channels contribute to the excitability of neurons (27). The two subunits of BK β are BK β 3 (KCNMB3) and BK β 4 (KCNMB4) (28). Therefore, KCNMB4 may be involved in the pathogenesis of NB through the voltage-gated Kv channel. In the present study, *KCNMB4* was involved in the regulation of action potential in neuron biological process.



Figure 4. Correlation analysis of the expression levels of *SOX2* and *MYCN*. Correlation coefficient= -0.83. Values were obtained relative to the control.

Based on these findings, it was hypothesized that *KCNMB4* is involved in the development of NB by the regulation of action potentials in neurons.

SOX2, located in chromosome 3q26.33, is a transcription factor which is involved in the regulation of stem cell properties (29,30). Previously, it has been reported that neural stem cell-like cells are isolated from certain types of neural cancer, including glioblastoma, medulloblastoma and NB (31,32). In addition, Das et al demonstrated that SOX2 is involved in the maintenance of undifferentiated stem cells by the regulation of all-trans retinoic acid, which induces the differentiation of NB cells. Furthermore, miR-340 has been found to be involved in the pathogenesis of NB by mediating the expression of SOX2 (33). Wang et al found that FoxM1 is involved in the tumorigenicity of aggressive NB cells by activating the expression of SOX2 (34). According to a report by Yang et al, the expression of OCT4 and SOX2 are involved in the progression of NB (35). These results indicate that SOX2 is important in the pathogenesis of NB. The results of the present study, which revealed that the expression of SOX2 was directly correlated with the expression of MYCN, were consistent with previous findings. Therefore, SOX2 may be involved in the pathology of NB through the regulation of MYCN.

In the present study, PPI network analysis indicated that GLI1 regulated SOX2 by the mediation of GLI3, and FOX also regulated the expression of SOX2 by the mediation of DCN. GLI1 and GLI3 are mediators of the SHH pathway, which has an effect on the early development of the central nervous system (36). In addition, SHH-GLI1 is involved in the self-renewal of cancer stem cells (37). Furthermore, Shahi et al reported that the expression of SMO and GLI3 are partially correlated in NB (38). These results suggest that GLI1 and GLI3 may be involved in the development of NB by the mediation of SOX2. In addition, FOX proteins are a conserved transcriptional regulator superfamily, and FOX family transcription factors are key in the progression of cancer (39). In NB, FOXR1, fused to MLL or PAFAH1B due to interstitial deletions, function as oncogenes (40). Santo et al demonstrated that events of intrachromosomal deletion/fusion at 11q23 activate the expression of FOXR1 in NB (41). This suggested that FOX may also be involved in the pathogenesis of NB by the regulation of SOX2. According to these findings, the present study hypothesized that FOS, GLI3 and GLI1 may regulate the expression of MYCN by the mediation of SOX2. Although the findings of the present study were consistent with previous observations that the FOS, GLI3 and GLI1 genes are involved in the pathogenesis of NB, the present study revealed that the FOS, GLI3 and GLI1 genes were involved in the ECM-receptor interaction pathway. Therefore, FOS, GLI3 and GLI1 may be involved in the ECM-receptor interaction pathway to contribute to the development of NB.

In conclusion, the present study identified 172 DEGs between the *MYCN* amplification group and the control group, including 137 downregulated and 35 upregulated DEGs. *KCNMB4* may contribute to the development of NB by the regulation of action potentials in neurons. *FOS*, *GL13* and *GL11* may be involved in the pathogenesis of NB by the regulation of *SOX2*, the expression of which was inversely correlated to the expression of *MYCN* in the NB cell lines. These findings have provided a novel opportunity to investigate the pathogenesis of NB, however, these results require confirmation by further investigations.

References

- Wang K, Diskin SJ, Zhang H, Attiyeh EF, Winter C, Hou C, Schnepp RW, Diamond M, Bosse K, Mayes PA, *et al*: Integrative genomics identifies LMO1 as a neuroblastoma oncogene. Nature 469: 216-220, 2011.
- Maris JM: Recent advances in neuroblastoma. N Engl J Med 362: 2202-2211, 2010.
- 3. Huang M and Weiss WA: Neuroblastoma and MYCN. Cold Spring Harb Perspect Med 3: a014415, 2013.
- 4. Nishira H, Toyoda Y, Tanaka Y, Ijiri R, Aida N, Takeuchi M, Ohnuma K, Kigasawa H, Kato K and Nishi T: Natural course of neuroblastoma detected by mass screening: S 5-year prospective study at a single institution. J Clin Oncol 18: 3012-3017, 2000.
- 5. De Bernardi B, Gerrard M, Boni L, Rubie H, Cañete A, Di Cataldo A, Castel V, Forjaz de Lacerda A, Ladenstein R, Ruud E, *et al*: Excellent outcome with reduced treatment for infants with disseminated neuroblastoma without MYCN gene amplification. J Clin Oncol 27: 1034-1040, 2009.
- Kaneko M, Tsuchida Y, Mugishima H, Ohnuma N, Yamamoto K, Kawa K, Iwafuchi M, Sawada T and Suita S: Intensified chemotherapy increases the survival rates in patients with stage 4 neuroblastoma with MYCN amplification. J Pediatr Hematol Oncol 24: 613-621, 2002.
- Tang XX, Zhao H, Kung B, Kim DY, Hicks SL, Cohn SL, Cheung NK, Seeger RC, Evans AE and Ikegaki N: The MYCN enigma: Significance of MYCN expression in neuroblastoma. Cancer Res 66: 2826-2833, 2006.
- Farina AR, Di Ianni N, Cappabianca L, Ruggeri P, Ragone M, Ianni G, Gulino A and Mackay AR: TrkAIII promotes microtubule nucleation and assembly at the centrosome in SH-SY5Y neuroblastoma cells, contributing to an undifferentiated anaplastic phenotype. Biomed Res Int 2013: 740187, 2013.
 Suenaga Y, Islam SM, Alagu J, Kaneko Y, Kato M, Tanaka Y,
- 9. Suenaga Y, Islam SM, Alagu J, Kaneko Y, Kato M, Tanaka Y, Kawana H, Hossain S, Matsumoto D, Yamamoto M, *et al*: NCYM, a Cis-antisense gene of MYCN, encodes a de novo evolved protein that inhibits GSK3β resulting in the stabilization of MYCN in human neuroblastomas. PLoS Genet 10: e1003996, 2014.
- Alderton GK: Neuroblastoma: A new gene that promotes NMYC activity. Nature Reviews Cancer 14: 155-155, 2014.
 Wu PY, Liao YF, Juan HF, Huang HC, Wang BJ, Lu YL, Yu IS,
- 11. Wu PY, Liao YF, Juan HF, Huang HC, Wang BJ, Lu YL, Yu IS, Shih YY, Jeng YM, Hsu WM and Lee H: Aryl hydrocarbon receptor downregulates MYCN expression and promotes cell differentiation of neuroblastoma. PLoS One 9: e88795, 2014.
- Fujita A, Sato JR, Rodrigues Lde O, Ferreira CE and Sogayar MC: Evaluating different methods of microarray data normalization. BMC Bioinformatics 7: 469, 2006.
- Smyth GK: Limma: Linear models for microarray data. In: Bioinformatics and computational biology solutions using R and Bioconductor Springer, pp397-pp420, 2005.
- Benjamini Y: Discovering the false discovery rate. J R Stat Soc Series B Stat Methodol 72: 405-416, 2010.
- 15. Benjamini Y and Hochberg Y: Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol: 289-300, 1995.
- Szekely GJ and Rizzo ML: Hierarchical clustering via joint between-within distances: Extending Ward's minimum variance method. Journal of Classification 22: 151-183, 2005.
- 17. Deza MM and Deza E: Encyclopedia of distances. Springer, 2009.
- Jiao X, Sherman BT, Huang da W, Stephens R, Baseler MW, Lane HC and Lempicki RA: DAVID-WS: A stateful web service to facilitate gene/protein list analysis. Bioinformatics 28: 1805-1806, 2012.
- Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P, Doerks T, Stark M, Muller J, Bork P, *et al*: The STRING database in 2011: Functional interaction networks of proteins, globally integrated and scored. Nucleic Acids Res 39: D561-D568, 2011.
- Smoot ME, Ono K, Ruscheinski J, Wang PL and Ideker T: Cytoscape 2.8: New features for data integration and network visualization. Bioinformatics 27: 431-432, 2011.
- Zhang B, Kirov S and Snoddy J: WebGestalt: An integrated system for exploring gene sets in various biological contexts. Nucleic Acids Res 33: W741-W748, 2005.
- 22. Wang J, Duncan D, Shi Z and Zhang B: WEB-based GEne SeT AnaLysis Toolkit (WebGestalt): Update 2013. Nucleic Acids Res 41: W77-W83, 2013.

- 23. Bieniasz M, Oszajca K, Eusebio M, Kordiak J, Bartkowiak J and Szemraj J: The positive correlation between gene expression of the two angiogenic factors: VEGF and BMP-2 in lung cancer patients. Lung Cancer 66: 319-326, 2009.
- 24. Bean BP: The action potential in mammalian central neurons. Nat Rev Neurosci 8: 451-465, 2007.
- 25. Hall MK, Cartwright TA, Fleming CM and Schwalbe RA: Importance of glycosylation on function of a potassium channel in neuroblastoma cells. PLoS One 6: e19317, 2011.
- 26. Leung YM, Huang CF, Chao CC, Lu DY, Kuo CS, Cheng TH, Chang LY and Chou CH: Voltage-gated K+ channels play a role in cAMP-stimulated neuritogenesis in mouse neuroblastoma N2A cells. J Cell Physiol 226: 1090-1098, 2011.
- 27. Manna I, Labate A, Mumoli L, Ferlazzo E, Aguglia U, Quattrone A and Gambardella A: Failure to confirm association of a polymorphism in KCNMB4 gene with mesial temporal lobe epilepsy. Epilepsy Res 106: 284-287, 2013.
- 28. Behrens R, Nolting A, Reimann F, Schwarz M, Waldschütz R and Pongs O: HKCNMB3 and hKCNMB4, cloning and characterization of two members of the large-conductance calcium-activated potassium channel beta subunit family. FEBS Lett 474: 99-106, 2000.
- 29. Gómez-Mateo Mdel C, Piqueras M, Påhlman S, Noguera R and Navarro S: Prognostic value of SOX2 expression in neuroblastoma. Genes Chromosomes Cancer 50: 374-377, 2011.
- 30. Pietras A, Gisselsson D, Ora I, Noguera R, Beckman S, Navarro S and Påhlman S: High levels of HIF-2alpha highlight an immature neural crest-like neuroblastoma cell cohort located in a perivascular niche. J Pathol 214: 482-488, 2008.
- 31. Melone MA, Giuliano M, Squillaro T, Alessio N, Casale F, Mattioli E, Cipollaro M, Giordano A and Galderisi U: Genes involved in regulation of stem cell properties: Studies on their expression in a small cohort of neuroblastoma patients. Cancer Biol Ther 8: 1300-1306, 2009.

- 32. Hansford LM, McKee AE, Zhang L, George RE, Gerstle JT, Thorner PS, Smith KM, Look AT, Yeger H, Miller FD, *et al*: Neuroblastoma cells isolated from bone marrow metastases contain a naturally enriched tumor-initiating cell. Cancer Res 67: 11234-11243, 2007.
- 33. Das S, Bryan K, Buckley PG, Piskareva O, Bray IM, Foley N, Ryan J, Lynch J, Creevey L, Fay J, *et al*: Modulation of neuroblastoma disease pathogenesis by an extensive network of epigenetically regulated microRNAs. Oncogene 32: 2927-2936, 2013.
- 34. Wang Z, Park HJ, Carr JR, Chen YJ, Zheng Y, Li J, Tyner AL, Costa RH, Bagchi S and Raychaudhuri P: FoxM1 in tumorigenicity of the neuroblastoma cells and renewal of the neural progenitors. Cancer Res 71: 4292-4302, 2011.
- 35. Yang S, Zheng J, Ma Y, Zhu H, Xu T, Dong K and Xiao X: Oct4 and Sox2 are overexpressed in human neuroblastoma and inhibited by chemotherapy. Oncol Rep 28: 186-192, 2012.
- 36. Zhu H and Lo HW: The human glioma-associated oncogene homolog 1 (Gli1) family of transcription factors in gene regulation and diseases. Curr Genomics 11: 238-245, 2010.
- 37. Clement V, Sanchez P, de Tribolet N, Radovanovic I and Ruiz i Altaba A: HEDGEHOG-GL11 signaling regulates human glioma growth, cancer stem cell self-renewal and tumorigenicity. Curr Biol 17: 165-172, 2007.
- Shahi MH, Lorente A and Castresana JS: Hedgehog signalling in medulloblastoma, glioblastoma and neuroblastoma. Oncol Rep 19: 681-688, 2008.
- Myatt SS and Lam EWF: The emerging roles of forkhead box (Fox) proteins in cancer. Nat Rev Cancer 7: 847-859, 2007.
- Katoh M, Igarashi M, Fukuda H and Nakagama H: Cancer genetics and genomics of human FOX family genes. Cancer Lett 328: 198-206, 2013.
- 41. Santo EE, Ebus ME, Koster J, Schulte JH, Lakeman A, van Sluis P, Vermeulen J, Gisselsson D, Øra I, Lindner S, *et al*: Oncogenic activation of FOXR1 by 11q23 intrachromosomal deletion-fusions in neuroblastoma. Oncogene 31: 1571-1581, 2012.