

The roles of microRNAs in neuroblastoma

Hong Mei, Zhen-Yu Lin, Qiang-Song Tong

Wuhan, China

Background: Neuroblastoma (NB) is the most common extracranial solid tumor in childhood and displays remarkable heterogeneity in clinical behaviors, ranging from spontaneous regression to rapid progression or resistance to multimodal treatment. Recent evidence has shown that microRNAs (miRNAs), a class of small non-coding RNAs, are involved in tumor development and progression. This article aimed to review recent advances in investigating the roles of miRNAs in NB.

Methods: We searched the PubMed/MEDLINE database for articles about the expression profile, functions and target genes of miRNAs in NB.

Results: We reviewed the most recent evidence regarding the functional roles of oncogenic and tumor suppressive miRNAs in NB and application of novel miRNA-based methods for diagnostic, prognostic and therapeutic purposes.

Conclusions: Deregulation of miRNAs is associated with the development and progression of NB, suggesting that miRNAs may serve as novel targets for the treatment of high-risk NB patients. However, their precise functions and underlying mechanisms still warrant further studies.

World J Pediatr 2014;10(1):10-16

Key words: microRNA;
neuroblastoma;
treatment

Introduction

Neuroblastoma (NB), the most common extracranial malignant solid tumor in childhood, accounts for 15% of all childhood cancer mortality, and presents remarkable heterogeneity in pathological, biological, genetic and clinical characteristics.^[1] Some NBs can spontaneously regress or differentiate into benign ganglioneuroma, whereas others present rapid progression or resistance to multimodal treatment.^[2-4] For patients with high-risk NB, many therapeutic approaches such as surgery, chemoradiotherapy, stem cell transplantation, and anti-disialoganglioside (GD2) immunotherapy combined with cytokines, fail to improve the clinical outcome.^[4] The estimated 5-year event-free survival rate of high-risk NB is lower than 50%, and its treatment remains challenging.

MicroRNAs (miRNAs) were first discovered in 1993 as a class of small non-protein-coding RNAs, ranging from 22 to 25 nucleotides in length.^[5] These small RNAs are conserved during evolution, and control gene expression at transcriptional and post-transcriptional levels in bacteria, viruses, plants, and animals. Moreover, investigations indicated that miRNAs were involved in carcinogenesis and aggressive progression of various tumors, including breast cancer,^[6] non-small cell lung cancer,^[7] prostate cancer,^[8] ovarian cancer,^[9] etc. More recently, miRNAs such as miR-17-92 cluster and miR-34a have been implicated in the pathogenesis of NB.^[1] The present review highlights recent findings and crucial roles of miRNAs in regulating the biological behavior of NB, which indicate the potential application of novel miRNA-based strategies for diagnostic, prognostic and therapeutic purposes.

Biogenesis of miRNAs and its regulation

miRNAs are the most widely studied small non-coding RNAs that negatively regulate gene expression in human cells.^[10] Biogenesis of miRNAs is a complex process. miRNAs are processed from primary precursor molecules (pri-miRNAs), which are transcribed from either introns of protein-coding genes or other non-coding regions of chromosomes.^[10] In the canonical

Author Affiliations: Department of Pediatric Surgery (Mei H, Tong QS), and Cancer Center (Lin ZY), Union Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

Corresponding Author: Qiang-Song Tong, PhD, MD, Department of Pediatric Surgery, Union Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China (Tel: 86-27-85726005; Email: qs_tong@hotmail.com)

doi: 10.1007/s12519-014-0448-2

©Children's Hospital, Zhejiang University School of Medicine, China and Springer-Verlag Berlin Heidelberg 2014. All rights reserved.

pathway, the pri-miRNAs are cleaved by RNase III endonuclease Drosha and double-stranded RNA-binding domain protein DiGeorge syndrome critical region gene 8 (DGCR8; also known as Pasha in *Drosophila melanogaster* and *Caenorhabditis elegans*) in the nucleus. The cleaved products are exported to cytoplasm where another RNase III endonuclease Dicer processes them into mature ~22 nt miRNA/miRNA* duplexes.^[10] One strand of this duplex is usually incorporated into the miRNA-induced silencing complex, and most animal miRNAs form imperfect base pairs with their target mRNAs, usually in the 3'-untranslated region (3'-UTR), leading to translational repression or deadenylation and degradation.^[10] Over one third of human genes are predicted to be directly targeted by miRNAs. Expression profiling studies indicate that most miRNAs are under the control of tissue-specific and/or developmental signaling.^[11]

Transcriptional control confers another important regulation of miRNA biogenesis. Numerous transcription factors, such as p53, MYC, myogenin and myoblast determination 1 (MYOD1), are shown to participate in the transcriptional control of miRNA genes.^[11] Enhancers, silencing elements, and chromatin modification also contribute to miRNA gene regulation. For instance, the miR-203 locus is frequently subjected to DNA methylation in hematopoietic tumors, but not in normal T lymphocytes.^[12] Consequently, the precise control of miRNA levels is crucial to maintain normal cellular function, and dysregulation of miRNA is often associated with human diseases, including cancers. Thus, the roles of miRNAs have emerged as a new research frontier for understanding the mechanisms of tumorigenesis.

Emerging roles of miRNAs in NB

miRNAs contribute to almost all aspects of tumor biology such as proliferation, apoptosis, differentiation, invasion, metastasis, and angiogenesis. Many miRNAs are found to be up- or down-regulated in cancer samples relative to their normal tissue counterparts.^[13] Aberrantly expressed miRNAs are also involved in the initiation and progression of pediatric NB as oncogenes or tumor suppressors (Fig.), and some particular miRNAs are considered as emerging potential candidates for improving the diagnosis, prognosis and therapies of human cancers. We will discuss the expression profile, potential functions, and target genes of different miRNAs in the tumorigenesis and aggressiveness of pediatric NB (Table).

Oncogenic miRNAs in NB

Different approaches have been applied to identify the

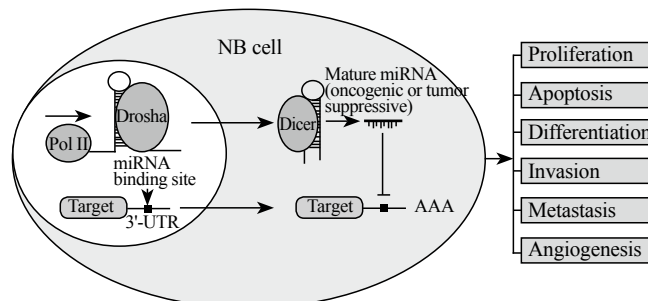


Fig. miRNAs as oncogenes or tumor suppressors in neuroblastoma. NB: neuroblastoma; miRNAs: microRNAs; Pol II: RNA polymerase II; 3'-UTR: 3'-untranslated region; AAA: poly-A tail of mature mRNA.

expression profiles of miRNAs in NB. In determining different expression profiles of miRNAs in low-, intermediate- and high-risk NB specimens, many miRNAs were found to be up-regulated in high-risk NB, including miR-18b, miR-20a, miR-22, miR-92a, miR-181a, miR-181a-2, miR-203, miR-373, miR-383, miR-422a, miR-876-5p, miR-1208, miR-1285, miR-1290, and miR-1291.^[40] Through establishing the heterotopic transplantation murine model of NB, Guo et al^[41] identified 35 up-regulated miRNAs in metastatic NB tumors, such as miR-24, miR-92b, miR-99b, miR-129-3p, miR-130b, miR-342-3p, miR-345, miR-483-3p, and miR-486-5p. The miR-17-92 polycistronic cluster, including miR-17-5p, miR-18a, miR-19a, miR-20a and miR-92, was highly expressed in NB tissues due to amplification of the miR-17-92 locus or direct transactivation by c-MYC/MYCN,^[14] and was a marker for poor survival of NB.^[15,16] Interestingly, although miR-17-92 was also up-regulated along with elevated MYC/MYCN levels in medulloblastoma (MB), another common malignant solid tumor in children, its expression was highest in MB subgroup with activation of the sonic hedgehog signaling pathway.^[42] In addition, MYCN-induced down-regulation of Dickkopf-3 (DKK3) in NB, a gene encoding an extracellular protein with presumed tumor suppressor activity, was caused by direct up-regulation of miR-17-92 components, which further contributed to the pleiotropic oncogenic effects of MYCN.^[17] However, no miRNA was identified to directly repress DKK3 in MB so far.^[43] Moreover, Schulte et al^[16] reported that oncogenic miR-181 was over-expressed in unfavorable NB. miR-21, a very well-known oncogenic miRNA in a variety of cancer types, promoted the proliferation and decreased the chemosensitivity of human NB cells.^[18] However, Buechner et al^[44] reported that inhibition of miR-21 did not affect the differentiation or proliferation of NB cells, suggesting that the precise biological and regulatory functions of miR-21 in NB still warrant further studies. In another study,^[19] miR-380-5p was

Table. Roles of oncogenic and tumor suppressive miRNAs (microRNAs) in neuroblastoma

miRNA	Expression patterns	Target genes	Potential function
Oncogenic			
miR-17-92 ^{[1,14-17]*}	Up-regulated in high-risk NB	DKK3, CDKN1A, BIM, ER- α , MEF2D	Increases proliferation, decreases apoptosis, and inhibits TGF- β signaling
miR-181 ^[16]	Over-expressed in unfavorable NB	ATM	NA
miR-21 ^[18]	Up-regulated in high-risk NB	PTEN	Promotes proliferation, and decreases sensitivity to cisplatin
miR-380-5p ^[19]	Up-regulated in high-risk NB	p53	Decreases apoptosis
miR-124 ^[20]	NA	AHR	Inhibits apoptosis and differentiation
miR-15a ^[21]	Up-regulated in metastatic NB	RECK	Promotes migration
Tumor suppressive			
miR-34a ^[1,22-24]	Down-regulated in high-risk NB	E2F3, BCL2, CCND1, CDK4, MYCN	Inhibits proliferation and induces apoptosis
miR-34c ^[1,22,23]	Down-regulated in high-risk NB	MYCN	Anti-proliferative effects
miR-410,487b ^[25]	Down-regulated in high-risk NB	NA	Biomarkers of relapse
miR-628 ^[26]	Expressed in favorable NB, absent in unfavorable NB	NA	NA
miR-542-5p ^[26,27]	Expressed in favorable NB, absent in unfavorable NB	GRIN3A, SH3GLB2, SNIP	Inhibits tumor growth, invasiveness and metastasis
let-7 ^[28]	Down-regulated in NB with MNA	MYCN	Inhibits proliferation and induces differentiation
miR-101 ^[28]	Down-regulated in NB with MNA	MYCN	Inhibits proliferation and clonogenic growth
miR-885-5p ^[29]	Down-regulated in NB	CDK2, MCM5	Inhibits cell cycle progression and survival
miR-27b ^[30]	Down-regulated in NB	PPAR γ	Inhibits tumor growth
miR-138 ^[31]	Down-regulated in NB	NA	Decreases viability and growth
miR-137 ^[32]	Down-regulated in NB	KDM1A	Inhibits proliferation and induces apoptosis
miR-204 ^[33]	Down-regulated in high-risk NB	BCL2, NTRK2	Increases sensitivity to cisplatin and etoposide
miR-10a/b ^[34]	Under-expressed in NB	NCOR2	Induces differentiation
miR-335 ^[35,36]	Down-regulated in NB	ROCK1, MAPK1, LRG1	Inhibits invasiveness and metastasis
miR-363 ^[35]	Down-regulated in NB	NA	Inhibits tumorigenesis and metastasis
miR-190 ^[37]	Down-regulated in high-risk NB	NEUROD1	Inhibits TrkB pathway and aggressive phenotypes
miR-9 ^[38]	Down-regulated in NB	MMP-14	Inhibits the invasion, metastasis, and angiogenesis
miR-145 ^[39]	Down-regulated in NB	HIF-2 α	Inhibits the growth, invasion, metastasis and angiogenesis

*: including miR-17-5p, miR-18a, miR-19a, miR-20a and miR-92. AHR: aryl hydrocarbon receptor; ATM: ataxia telangiectasia mutated; BCL2: B-cell CLL/lymphoma 2; BIM: BCL2-like 11; CCND1: cyclin D1; CDK2: cyclin-dependent kinase; CDK4: cyclin-dependent kinase 4; CDKN1A: cyclin-dependent kinase inhibitor 1A; DDK3: Dickkopf-3; E2F3: transcription factor 3; ER- α : estrogen receptor 1 (alpha); GRIN3A: glutamate receptor, ionotropic, N-methyl-D-aspartate 3A; HIF-2 α : hypoxia-inducible factor 2 alpha; KDM1A: lysine-specific demethylase 1; LRG1: leucine-rich alpha-2-glycoprotein 1; MAPK1: mitogen-activated protein kinase 1; MCM5: mini-chromosome maintenance protein; MEF2D: myocyte enhancer factor 2D; miRNA: microRNA; MMP-14: matrix metalloproteinase 14; MNA: MYCN amplification; MYCN: v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog; NA: not available; NB: neuroblastoma; NCOR2: nuclear receptor corepressor 2; NEUROD1: neuronal differentiation 1; NTRK2: neurotrophic tyrosine kinase receptor type 2; PPAR γ : peroxisome proliferators-activated receptor γ ; PTEN: phosphatase and tensin homolog; RECK: reversion-inducing cysteine-rich protein with Kazal motifs; ROCK1: Rho-associated coiled-coil containing protein kinase 1; SH3GLB2: SH3-domain GRB2-like endophilin B2; SNIP: SNAP25-interacting protein; TGF- β : transforming growth factor beta; TrkB: tyrosine kinase B.

highly expressed in NB to repress p53-mediated apoptosis, and was associated with poor outcome in NB with MYCN amplification. A recent study^[20] showed that miR-124 inhibited the apoptosis and differentiation of NB cells through targeting aryl hydrocarbon receptor (AHR). miR-15a promoted the migration of NB cells through targeting reversion-inducing cysteine-rich protein with Kazal motifs and regulating downstream matrix metalloproteinase 9 (MMP-9) expression,^[21] indicating the importance of miR-15a-RECK-MMP-9 axis in NB progression.

Tumor suppressive miRNAs in NB

Bienertova-Vasku et al^[40] reported that certain miRNAs

(miR-30b, miR-146a, miR-190, miR-204, miR-215, miR-299-5p, miR-362, miR-382, miR-411, miR-424, miR-425, miR-487b, miR-532, miR-629, miR-656, miR-660, miR-668, miR-744, miR-758, miR-873, miR-885-5p, and miR-1197) were down-regulated in high-risk NB. In addition, miR-30c,^[41] miR-149,^[41] miR-195,^[45,46] miR-324-5p,^[47] and miR-331^[45] were also identified as down-regulated miRNAs in high-risk NB, and loss of their expression was associated with unfavorable prognosis. Moreover, Gattolliat et al^[25] found that miR-410 and miR-487b were significantly down-regulated in high-risk NB, and their expression was significantly associated with disease-free survival of non-MYCN-amplified NB, indicating their roles as biomarkers of relapse in favorable NB. Potential tumor suppressive miR-542-5p and miR-628 are expressed

in favorable NB and virtually absent in unfavorable NB.^[26] In accordance with these findings, Bray et al^[27] reported that the lower expression of miR-542-5p is highly associated with poor patient survival, suggesting a putative tumor suppressive function.

Subsequent studies have confirmed the tumor suppressive roles and target genes of many miRNAs in regulating the proliferation and apoptosis of NB. Among these tumor suppressive miRNAs in NB, miR-34a has been extensively investigated. The miR-34 family members, miR-34a and miR-34c, have been shown to target the 3'-UTR of MYCN and inhibit the growth of NB cell lines with 1p36 hemizygous deletion.^[22,23] In addition, other genes involved in cell proliferation or apoptosis were targeted by miR-34a, including E2F transcription factor 3, B-cell CLL/lymphoma 2, cyclin D1 (CCND1) and cyclin-dependent kinase 4 (CDK4).^[11] Chen et al^[24] also reported that CDK1 inhibition-induced death of NB cells was exerted through the miR-34a-MYCN-survivin pathway. However, miR-34a was not differentially expressed in favorable versus unfavorable NB.^[26] The tumor suppressive let-7 family members (let-7e and miR-202) and miR-101 targeted the proto-oncogene MYCN, and inhibited the proliferation and clonogenic growth of MYCN-amplified NB cells.^[28] Over-expression of miR-7-1 was found to increase the efficacy of green tea polyphenols by inducing apoptosis in NB cells.^[48] Moreover, miR-885-5p was down-regulated due to loss of the 3p25.3 region in NB, and played a tumor suppressive role in cell cycle progression and survival of NB cells through targeting CDK2 and mini-chromosome maintenance protein.^[29] miR-27b was found to target peroxisome proliferators-activated receptor γ and inhibit the growth of NB cells.^[30] Meanwhile, miR-137 played a tumor suppressive role in NB via down-regulation of lysine-specific demethylase 1, which was strongly expressed and correlated with poor prognosis of NB patients.^[32] miR-204 was confirmed to directly target the 3'-UTR of BCL2 and neurotrophic tyrosine kinase receptor type 2, and ectopic miR-204 expression significantly increased the sensitivity of NB cells to cisplatin and etoposide *in vitro*, suggesting it as a novel tumor suppressor of NB.^[33]

Several miRNAs have also been identified to regulate the differentiation of NB. miR-10a and miR-10b were demonstrated as potent differentiation inducers of NB cells via targeting nuclear receptor corepressor 2 (NCOR2), a corepressor of gene transcription.^[34] By using microarray profiling in combination with real-time PCR, Chen et al^[49] showed that miR-7 and miR-214 were specifically expressed during the differentiation of NB cells, and controlled neurite outgrowth *in vitro*.

Since invasion, metastasis, and angiogenesis are the leading causes of death in NB patients, a series of studies^[35-39,50] have been conducted to elucidate the roles of miRNAs in the aggressiveness of this disease. Qiao et al^[35] reported that miR-335 and miR-363 regulated the gastrin-releasing peptide receptor signaling-mediated tumorigenesis and metastatic potential of NB, whereas miR-335 was also found to suppress the invasiveness of NB cells by directly targeting the non-canonical TGF- β signaling pathway members Rho-associated coiled-coil containing protein kinase 1, mitogen-activated protein kinase 1, and putative member leucine-rich alpha-2- glycoprotein 1.^[36] Loss of miR-190 expression was demonstrated to lead to activation of the tyrosine kinase B pathway and aggressive phenotypes of NB via directly targeting neuronal differentiation 1.^[37] In addition, the let-7 family, miR-7, and miR-338-3p exerted anti-metastatic functions in NB.^[50] Our studies have shown that ectopic expression of miR-9^[38] and miR-145^[39] inhibited the growth, invasion, metastasis and angiogenesis of NB cells through targeting MMP-14 and hypoxia-inducible factor 2 alpha, respectively.

Potential clinical application of miRNAs in NB

In the past few years, it has been established that miRNA panels are helpful for the diagnosis and prognosis of cancers. Following the quick development of powerful miRNA techniques, such as miRNA microarrays, specific quantitative PCR of miRNA, bead-based miRNA profiling, and antisense technologies, miRNAs are expected to have a significant effect on clinical oncology in the next decade, and might be potential novel targets for the treatment of tumors, including NB. Since miRNAs are key factors that regulate many cellular processes, and are aberrantly expressed in tumor specimens relative to normal tissues, it is rational to apply them as valuable tools in cancer diagnosis. Conventional methods, such as RNase protection assay, Northern blot, and quantitative reverse transcription PCR, have been confirmed efficient in detecting the miRNA levels in NB tissues.^[13] In addition, miRNA markers are proposed as useful tools for the prognostic evaluation of NB. For example, Lin et al^[45] found 27 miRNAs that could clearly distinguish low- from high-risk patients, indicating that miRNA expression profile was significantly correlated with biological and clinical features of NB. Recently, miRNome analyses have become easier by microarray techniques, which are better at predicting the type, stage and pathological features of tumors than mRNA expression profile.

Different studies have shown that a number of miRNAs affect the growth, invasion and metastases of NB cells *in vitro* and *in vivo*. Consequently, the biology of NB cells can be controlled by manipulating miRNA levels. Over-expression or silencing of particular miRNA can be achieved in several ways. miRNAs can be up-regulated by synthetic mimics such as small interfering RNA-like oligoribonucleotide duplex or chemically modified oligoribonucleotide, and can be inhibited by modified antisense oligonucleotides.^[13] Transfection of miR-338-3p mimics into rat NB cells resulted in significant decreased levels of its host gene apoptosis-associated tyrosine kinase.^[51] Targeted delivery of miR-34a using anti-GD2-coated nanoparticles resulted in increased apoptosis and decreased growth and angiogenesis of NB cells.^[52] In addition, Chakrabarti et al^[31] reported that over-expression of miR-138 through direct transfection of mimics was more powerful than transfection of short hairpin RNA plasmid for decreasing the viability and colony formation capability of NB cell lines. Huang et al^[20] validated that knockdown of miR-124 facilitated the differentiation, cell cycle arrest and apoptosis of NB cells. These findings suggest that some special miRNAs can serve as potential therapeutic targets of human NB.

Some clinical trials are undergoing to confirm the roles of miRNAs in human cancers, and the results of which might help us in identifying prognostic factors to stratify the patients into risk categories. The results of Köberle et al^[53] indicated that serum miR-122 and miR-1 levels were correlated with clinical parameters of hepatocellular carcinoma (HCC) patients and might therefore improve the predictive value of classical HCC staging scores. A prospective cohort study (ClinicalTrials.gov, Identifier: NCT01612871) is undertaking to evaluate the values of a panel of circulating miRNA markers in identifying breast cancer patients who are most likely to respond well to neoadjuvant and adjuvant chemotherapy. A placebo-controlled, double-blind, randomized, dose escalating trial (ClinicalTrials.gov, Identifier: NCT00688012) in healthy men also evaluates the safety, tolerability, pharmacokinetics and pharmacodynamics of miR-122 inhibitor SPC3649, representing the first human clinical trial of miRNA inhibitor.

Taken together, preliminary results have suggested that miRNAs are useful for the diagnosis, prognosis and therapy of NB. Targeting strategies for particular miRNAs have been developed and assessed for their safety and efficacy in NB *in vivo* and *in vitro*. However, our understanding of the functions and gene regulatory networks of miRNAs in NB is still limited, further elucidation of their roles and extensive preclinical research are necessary before the clinical application.

Conclusions

NB is the most common extracranial solid tumor in childhood, and displays remarkable heterogeneity in clinical behavior. It has been currently established that deregulation of miRNAs is associated with the development and progression of NB. Although the two major pediatric malignant solid tumors, NB and MB, share some common miRNA profiles, they are quite different in target genes, regulatory mechanisms or potential functions, which might provide a novel way to improve the diagnosis and prognosis of NB. The development of novel miRNA-based therapeutic strategies that target the pathways responsible for malignant transformation and progression of NB is necessary, especially for the high-risk category.

Funding: This study was supported by grants from the National Natural Science Foundation of China (No. 30772359, No. 81071997, No. 81072073, No. 81272779, No. 81372667), Program for New Century Excellent Talents in University (NCET-06-0641), Scientific Research Foundation for the Returned Overseas Chinese Scholars (2008-889), and Research Fund for the Central University (2012QN224, 2013ZHYX003).

Ethical approval: Not needed.

Competing interest: All authors disclosed no conflict of interest.

Contributors: Mei H and Lin ZY contributed equally to the work. Mei H and Lin ZY drafted the initial manuscript, and approved the final manuscript as submitted. Tong QS reviewed and revised the manuscript, and approved the final manuscript as submitted.

References

- 1 Stallings RL. MicroRNA involvement in the pathogenesis of neuroblastoma: potential for microRNA mediated therapeutics. *Curr Pharm Des* 2009;15:456-462.
- 2 Brodeur GM. Neuroblastoma: biological insights into a clinical enigma. *Nat Rev Cancer* 2003;3:203-216.
- 3 Park JR, Eggert A, Caron H. Neuroblastoma: biology, prognosis, and treatment. *Hematol Oncol Clin North Am* 2010;24:65-86.
- 4 Park JR, Bagatell R, London WB, Maris JM, Cohn SL, Mattay KM, et al. Children's Oncology Group's 2013 blueprint for research: Neuroblastoma. *Pediatr Blood Cancer* 2013;60:985-993.
- 5 Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 1993;75:843-854.
- 6 Schwarzenbacher D, Balic M, Pichler M. The role of microRNAs in breast cancer stem cells. *Int J Mol Sci* 2013;14:14712-14723.
- 7 Xie Y, Todd NW, Liu Z, Zhan M, Fang H, Peng H, et al. Altered miRNA expression in sputum for diagnosis of non-small cell lung cancer. *Lung Cancer* 2010;67:170-176.
- 8 Gandellini P, Giannoni E, Casamichela A, Taddei ML, Callari M, Piovan C, et al. miR-205 hinders the malignant interplay between prostate cancer cells and associated fibroblasts. *Antioxid Redox Signal* 2013 Sep 17.
- 9 Di Leva G, Croce CM. The role of microRNAs in the

- tumorigenesis of ovarian cancer. *Front Oncol* 2013;3:153.
- 10 Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009;136:215-233.
 - 11 Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol* 2009;10:126-139.
 - 12 Bueno MJ, Pérez de Castro I, Gómez de Cedrón M, Santos J, Calin GA, Cigudosa JC, et al. Genetic and epigenetic silencing of microRNA-203 enhances ABL1 and BCR-ABL1 oncogene expression. *Cancer Cell* 2008;13:496-506.
 - 13 Lee YS, Dutta A. MicroRNAs in cancer. *Annu Rev Pathol* 2009;4:199-227.
 - 14 Mestdagh P, Fredlund E, Pattyn F, Schulte JH, Muth D, Vermeulen J, et al. MYCN/c-MYC-induced microRNAs repress coding gene networks associated with poor outcome in MYCN/c-MYC-activated tumors. *Oncogene* 2010;29:1394-1404.
 - 15 Mestdagh P, Boström AK, Impens F, Fredlund E, Van Peer G, De Antonellis P, et al. The miR-17-92 microRNA cluster regulates multiple components of the TGF- β pathway in neuroblastoma. *Mol Cell* 2010;40:762-773.
 - 16 Schulte JH, Marschall T, Martin M, Rosenstiel P, Mestdagh P, Schlierf S, et al. Deep sequencing reveals differential expression of microRNAs in favorable versus unfavorable neuroblastoma. *Nucleic Acids Res* 2010;38:5919-5928.
 - 17 De Brouwer S, Mestdagh P, Lambert I, Pattyn F, De Paep A, Westermann F, et al. Dickkopf-3 is regulated by the MYCN-induced miR-17-92 cluster in neuroblastoma. *Int J Cancer* 2012;130:2591-2598.
 - 18 Chen Y, Tsai YH, Fang Y, Tseng SH. Micro-RNA-21 regulates the sensitivity to cisplatin in human neuroblastoma cells. *J Pediatr Surg* 2012;47:1797-1805.
 - 19 Swarbrick A, Woods SL, Shaw A, Balakrishnan A, Phua Y, Nguyen A, et al. miR-380-5p represses p53 to control cellular survival and is associated with poor outcome in MYCN-amplified neuroblastoma. *Nat Med* 2010;16:1134-1140.
 - 20 Huang TC, Chang HY, Chen CY, Wu PY, Lee H, Liao YF, et al. Silencing of miR-124 induces neuroblastoma SK-N-SH cell differentiation, cell cycle arrest and apoptosis through promoting AHR. *FEBS Lett* 2011;585: 3582-3586.
 - 21 Xin C, Buhe B, Hongting L, Chuanmin Y, Xiwei H, Hong Z, et al. MicroRNA-15a promotes neuroblastoma migration by targeting reversion-inducing cysteine-rich protein with Kazal motifs (RECK) and regulating matrix metalloproteinase-9 expression. *FEBS J* 2013;280:855-866.
 - 22 Tivnan A, Tracey L, Buckley PG, Alcock LC, Davidoff AM, Stallings RL. MicroRNA-34a is a potent tumor suppressor molecule in vivo in neuroblastoma. *BMC Cancer* 2011;11:33.
 - 23 Cole KA, Attiyeh EF, Mosse YP, Laquaglia MJ, Diskin SJ, Brodeur GM, et al. A functional screen identifies miR-34a as a candidate neuroblastoma tumor suppressor gene. *Mol Cancer Res* 2008;6:735-742.
 - 24 Chen Y, Tsai YH, Tseng SH. Inhibition of cyclin-dependent kinase 1-induced cell death in neuroblastoma cells through the microRNA-34a-MYCN-survivin pathway. *Surgery* 2013;153:4-16.
 - 25 Gattolliat CH, Thomas L, Ciafrè SA, Meurice G, Le Teuff G, Job B, et al. Expression of miR-487b and miR-410 encoded by 14q32.31 locus is a prognostic marker in neuroblastoma. *Br J Cancer* 2011;105:1352-1361.
 - 26 Schulte JH, Marschall T, Martin M, Rosenstiel P, Mestdagh P, Schlierf S, et al. Deep sequencing reveals differential expression of microRNAs in favorable versus unfavorable neuroblastoma. *Nucleic Acids Res* 2010;38:5919-5928.
 - 27 Bray I, Tivnan A, Bryan K, Foley NH, Watters KM, Tracey L, et al. MicroRNA-542-5p as a novel tumor suppressor in neuroblastoma. *Cancer Lett* 2011;303:56-64.
 - 28 Buechner J, Tømte E, Haug BH, Henriksen JR, Løkke C, Flægstad T, et al. *Br J Cancer* 2011;105:296-303.
 - 29 Afanasyeva EA, Mestdagh P, Kumps C, Vandesompele J, Ehemann V, Theissen J, et al. MicroRNA miR-885-5p targets CDK2 and MCM5, activates p53 and inhibits proliferation and survival. *Cell Death Differ* 2011;18:974-984.
 - 30 Lee JJ, Drakaki A, Iliopoulos D, Struhl K. MiR-27b targets PPAR[γ] to inhibit growth, tumor progression and the inflammatory response in neuroblastoma cells. *Oncogene* 2012;31:3818-3825.
 - 31 Chakrabarti M, Banik NL, Ray SK. miR-138 overexpression is more powerful than hTERT knockdown to potentiate apigenin for apoptosis in neuroblastoma in vitro and in vivo. *Exp Cell Res* 2013;319:1575-1585.
 - 32 Althoff K, Beckers A, Odersky A, Mestdagh P, Köster J, Bray IM, et al. MiR-137 functions as a tumor suppressor in neuroblastoma by downregulating KDM1A. *Int J Cancer* 2013;133:1064-1073.
 - 33 Ryan J, Tivnan A, Fay J, Bryan K, Meehan M, Creevey L, et al. MicroRNA-204 increases sensitivity of neuroblastoma cells to cisplatin and is associated with a favourable clinical outcome. *Br J Cancer* 2012;107:967-976.
 - 34 Foley NH, Bray I, Watters KM, Das S, Bryan K, Bernas T, et al. MicroRNAs 10a and 10b are potent inducers of neuroblastoma cell differentiation through targeting of nuclear receptor corepressor 2. *Cell Death Differ* 2011;18:1089-1098.
 - 35 Qiao J, Lee S, Paul P, Theiss L, Tiao J, Qiao L, et al. miR-335 and miR-363 regulation of neuroblastoma tumorigenesis and metastasis. *Surgery* 2013;154:226-233.
 - 36 Lynch J, Fay J, Meehan M, Bryan K, Watters KM, Murphy DM, et al. MiRNA-335 suppresses neuroblastoma cell invasiveness by direct targeting of multiple genes from the non-canonical TGF- β signalling pathway. *Carcinogenesis* 2012;33:976-985.
 - 37 Slaby O. MiR-190 leads to aggressive phenotype of neuroblastoma through indirect activation of TrkB pathway. *Med Hypotheses* 2013;80:325-326.
 - 38 Zhang H, Qi M, Li S, Qi T, Mei H, Huang K, et al. microRNA-9 targets matrix metalloproteinase 14 to inhibit invasion, metastasis, and angiogenesis of neuroblastoma cells. *Mol Cancer Ther* 2012;11:1454-1466.
 - 39 Zhang H, Pu J, Qi T, Qi M, Yang C, Li S, et al. MicroRNA-145 inhibits the growth, invasion, metastasis and angiogenesis of neuroblastoma cells through targeting hypoxia-inducible factor 2 alpha. *Oncogene* 2012 Dec 10.
 - 40 Biernertova-Vasku J, Mazanek P, Hezova R, Curdova A, Nekvindova J, Kren L, et al. Extension of microRNA expression pattern associated with high-risk neuroblastoma. *Tumor Biol* 2013;34:2315-2319.
 - 41 Guo J, Dong Q, Fang Z, Chen X, Lu H, Wang K, et al. Identification of miRNAs that are associated with tumor metastasis in Neuroblastoma. *Cancer Biol Ther* 2010;9:446-452.
 - 42 Northcott PA, Fernandez-L A, Hagan JP, Ellison DW, Grajkowska W, Gillespie Y, et al. The miR-17/92 polycistron is up-regulated in sonic hedgehog-driven medulloblastomas and induced by N-myc in sonic hedgehog-treated cerebellar neural precursors. *Cancer Res* 2009;69:3249-3255.
 - 43 Valdora F, Banelli B, Stigliani S, Pfister SM, Moretti S, Kool M, et al. Epigenetic silencing of DKK3 in medulloblastoma. *Int J Mol Sci* 2013;14:7492-7505.

- 44 Buechner J, Henriksen JR, Haug BH, Tømte E, Flaegstad T, Einvik C. Inhibition of mir-21, which is up-regulated during MYCN knockdown-mediated differentiation, does not prevent differentiation of neuroblastoma cells. *Differentiation* 2011;81:25-34.
- 45 Lin RJ, Lin YC, Chen J, Kuo HH, Chen YY, Diccianni MB, et al. microRNA signature and expression of Dicer and Drosha can predict prognosis and delineate risk groups in neuroblastoma. *Cancer Res* 2010;70:7841-7850.
- 46 De Preter K, Mestdagh P, Vermeulen J, Zeka F, Naranjo A, Bray I, et al. miRNA Expression profiling enables risk stratification in archived and fresh neuroblastoma tumor samples. *Clin Cancer Res* 2011;17:7684-7692.
- 47 Buckley PG, Alcock L, Bryan K, Bray I, Schulte JH, Schramm A, et al. Chromosomal and microRNA expression patterns reveal biologically distinct subgroups of 11q- neuroblastoma. *Clin Cancer Res* 2010;16:2971-2978.
- 48 Chakrabarti M, Ai W, Banik NL, Ray SK. Overexpression of miR-7-1 Increases efficacy of green tea polyphenols for induction of apoptosis in human malignant neuroblastoma SH-SY5Y and SK-N-DZ cells. *Neurochem Res* 2013;38:420-432.
- 49 Chen H, Shalom-Feuerstein R, Riley J, Zhang SD, Tucci P, Agostini M, et al. miR-7 and miR-214 are specifically expressed during neuroblastoma differentiation, cortical development and embryonic stem cells differentiation, and control neurite outgrowth in vitro. *Biochem Biophys Res Commun* 2010;394:921-927.
- 50 Zhang H, Li Y, Lai M. The microRNA network and tumor metastasis. *Oncogene* 2009;29:937-948.
- 51 Kos A, Olde Loohuis NF, Wiczorek ML, Glennon JC, Martens GJ, Kolk SM, et al. A potential regulatory role for intronic microRNA-338-3p for its host gene encoding apoptosis-associated tyrosine kinase. *PLoS One* 2012;7:e31022.
- 52 Tivnan A, Orr WS, Gubala V, Nooney R, Williams DE, McDonagh C, et al. Inhibition of neuroblastoma tumor growth by targeted delivery of microRNA-34a using anti-disialoganglioside GD2 coated nanoparticles. *PLoS One* 2012;7:e38129.
- 53 Köberle V, Kronenberger B, Pleli T, Trojan J, Imelmann E, Peveling-Oberhag J, et al. Serum microRNA-1 and microRNA-122 are prognostic markers in patients with hepatocellular carcinoma. *Eur J Cancer* 2013;49:3442-3449.

Received September 24, 2013

Accepted after revision December 26, 2013