JBUON 2020; 25(4): 2105-2109
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

Expression of miR-140-5p and miR-370 in nephroblastoma and its effect on cell proliferation

Huiqing Li[1], Xuehui Wang[2]

[1]Department of Nephrology, First Affiliated Hospital of Qiqihar Medical College, Qiqihar 161400, P.R.China. [2]Department of Nephrology, General Hospital of the General Administration of Agriculture and Reclamation of Heilongjiang, Harbin 150088, P.R.China.

Summary

Purpose: *This study aimed to explore the expression changes of microRNA (miR)-140-5p and miR-370 in nephroblastoma and its effect on the proliferative ability of nephroblastoma WT_CLS1 and SK-NEP-1 cells.*

Methods: *The expression levels of miR-140-5p and miR-370 was detected by quantitative real-time PCR (qRT-PCR) in cancer tissues and normal adjacent tissues which were collected from 60 patients with nephroblastoma. Expression vectors of miR-140-5p and miR-370 were constructed, and transient transfection of human nephroblastoma cell lines WT_CLS1 and SK-NEP-1 was carried out in vitro. There were three groups of the two genes: Blank cell group (blank group); Gene transfection group (miR-490-5p group, miR-370 group); and No-load transfection group (NLTF group). The proliferative ability of WT_CLS1 and SK-NEP-1 cells was detected by MTT assay.*

Results: *The results of miR-140-5p and miR-370 detected by qRT-PCR showed that in cancer tissues the expression level of miR-140-5p was significantly lower than that in adjacent tissues, and the level of miR-370 was significantly higher than that in adjacent tissues, and the difference was statistically significant ($p<0.001$). The proliferation of WT_CLS1 and SK-NEP-1 cells in miR-140-5p group was significantly lower than that in NLTF group ($p<0.05$), while the proliferation of WT_CLS1 and SK-NEP-1 cells in miR-370 group was significantly higher than that in NLTF group ($p<0.05$).*

Conclusion: *miR-140-5p is lowly expressed and miR-370 is highly expressed in nephroblastoma tissues; miR-370 can promote the proliferation of WT-CLS1 cells in nephroblastoma, and miR-140-5p can inhibit their proliferation and it may become a new target for the treatment of nephroblastoma in the future.*

Key words: *miR-140-5p, miR-370, nephroblastoma, cell proliferation, WT_CLS1, SK-NEP-1*

Introduction

Nephroblastoma is an embryonic malignant solid tumor that mostly occurs in children [1]. According to the statistical results of Gooskens et al [2,3], 98% of cases of nephroblastoma occurred under the age of 10, mostly under 3 years old, and the incidence of nephroblastoma in children under 15 decreased significantly, while the incidence of nephroblastoma was lower in adults and there was no significant difference between males and females [4]. The clinical manifestations are unilateral or bilateral abdominal mass, abdominal distension accompanied by hypertension, headache, hemoptysis and other symptoms [5]. In recent years, rapid progress has been made in the diagnosis and treatment of nephroblastoma [6]. The results of Kumar et al [7] showed that the overall 4-year survival rate of American nephroblastoma patients was 95%, and the 4-year tumor-free survival rate was 88%. However, there are still some patients in whom control of tumor proliferation is

Corresponding author: Dr. Xuehui Wang, MD. Department of Nephrology, General Hospital of the General Administration of Agriculture and Reclamation of Heilongjiang, No.235 Hashuang Rd, Harbin 150088, P.R.China.
E-mail: wangxh06n@163.com
Received: 12/03/2019; Accepted: 21/04/2019

not achieved because of misdiagnosis or improper treatment, which directly affects the patient's condition [8,9].

The etiology and pathogenesis of nephroblastoma are very complicated, and the proliferative mechanism of nephroblastoma has not been fully understood at present [10]. In recent years, miRs have become a hot research and analysis topic. MiRs are a group of non-coding RNAs consisting of about 19-25 nucleotides in length, which can regulate the expression of multiple genes after transcription and thus play an important role as oncogenes or anti-oncogenes. MiRs also play an important role in ontogeny [11,12]. At present, it has been reported that the increase of miR-370 is closely related to breast cancer, lung cancer, prostate cancer and many other malignant tumors, and miR-140-5p can promote cell proliferation and inhibit apoptosis in various tumor cell lines [13,14]. Therefore, by analyzing the expression of miR-140-5p and mir-370 in nephroblastoma and its proliferation in WT_CLS1 and SK-NEP-1 cells, researchers further clarified the role of these two miRs in nephroblastoma and provided reliable and effective reference for targeted therapeutic gene in this study.

Methods

Clinical data of patients

60 patients, including 34 males and 26 females with age ranging of 1-37 years (average 10.37±7.61 years), with nephroblastoma diagnosed in First Affiliated Hospital of Qiqihar Medical College from January 2015 to December 2017 were selected in this study. Cancer tissues and normal adjacent tissues of patients were obtained (Table 1).

Main materials

Human nephroblastoma cell line WT_CLS1 and SK-NEP-1 was purchased from Fujian StemEry Hematopoietic Technology Co. Ltd, Fujian, China; Reverse transcription kit Fexmentas K1622 was purchased from Beijing Think-far Technology Co., Ltd, China; TransScript Green Two-Step qRT-PCR SuperMix was purchased from TransGen Biotech (Beijing, China); Nuclease-Free Water was purchased from Xiamen Research and Science Biotechnology Co., Ltd., China; RT-PCR kit was purchased from China Thermo Fisher Scientific Co., Ltd; RNA extraction using TRIzol Reagent was purchased from Shanghai Pufei Biotechnology Co., Ltd, China; Nanodrop 2000 UV spectrophotometer was purchased from Thermo Scientific, Boston, USA; Bovine fetal serum (BFS), methyl thiazolyl tetrazolium (MTT), and dimethyl sulfoxide (DMSO), were purchased from Sigma-Aldrich Biotechnology Co., Ltd, New York, USA; RPMI-1640 medium was purchased from Hyclone Biotechnology Co., Ltd, Logan, USA; Lipofectamine 3000 transfection reagent was purchased from China Thermo Fisher Scientific Co., Ltd.

Cell culture and transfection, grouping

Human nephroblastoma cell lines WT_CLS1 and SK-NEP-1 were added to RPMI-1640 medium containing 10% FBS and incubated in an incubator containing 5% CO_2 at 37°C. When cultured to the third generation, it could be used for research. The cells in logarithmic growth phase were taken and the medium was replaced by FBS free medium 1 h before transfection. When the total cell solubility was about 90%, the transfection with Lipofectamine 3000 kit was carried out in strict accordance with the instructions of the transfection kit. After transfection, it was incubated in an incubator containing 5% CO_2 at 37°C for 48 h. Then, qPT-PCR was used to detect the expression of miR-140-5p and mir-370, and the results of transfection were analyzed. Transfection of miR-140-5p and mir-370 was according to instructions of Liposome Lipofectamine 3000 for cell transfection, and they were divided into miR-140-5p group and blank group (NLTF group), miR-370 group and blank group (NLTF group) accordingly.

qRT-PCR detection

Trizol reagent was used to extract the spare nephroblastoma cancer tissues and adjacent tissues according to the instructions of RNA extraction kit. The specific steps were strictly in accordance with the instructions.

Table 1. Basic data of 60 patients with nephroblastoma

Data	*n=60* *n (%)*
Age, years	
<10	48 (80.00)
≥10	12 (20.00)
Sex	
Male	34 (56.67)
Female	26 (43.33)
Abdominal distention	
Yes	41 (68.33)
No	19 (31.67)
With hypertension	
Yes	32 (53.33)
No	28 (46.67)
Pathological diagnosis types	
Clear cell carcinoma	29 (48.33)
Granular cell carcinoma	18 (30.00)
Undifferentiated cell carcinoma	13 (21.67)
Stages	
I	8 (13.33)
II	15 (25.00)
III	14 (23.33)
IV	17 (28.33)
V	6 (10.00)

The concentration and purity of the extracted RNA was detected by nanodrop 2000 UV spectrophotometer, and the integrity of the extracted RNA was detected by 1% denaturing agarose gel electrophoresis. MiR-140-5p and mir-370 reverse transcription was performed according to the kit instructions. The PCR reaction was carried out and the reaction system was configured strictly according to the qRT-PCR kit. One μg of RNA was added to the reverse transcription system, and the reverse transcription procedure was at 37°C for 15 min, 85°C for 5 s, and 4°C for 10 min. qRT-PCR: The reverse transcription product cDNA was used as template to amplify on the amplifier. PCR reaction system: 1 μl of DNA, 0.4 μl of upstream and downstream primers, 0.4 μl of passive reference dye (50x) (optional), 0.4 μl of 2xTransScript® Tip Green qRT-PCR SuperMix 10 μl, and 20 μl of the system was completed by nuclease-free water. The experiment was carried out in triplicate. The reaction conditions were: pre-denaturation at 94°C for 30 s; denaturation at 94°C for 5 s; annealing at 50-60°C for 15 s and extension at 72°C for 10 s for a total of 30 cycles, using U6 as internal parameter and using the $2^{-\Delta Ct}$ method to calculate the relative expression level. For the sequence of primers see Table 2.

MTT assay

The transfected human nephroblastoma cells WT-CLS1 and SK-NEP-1 were prepared into single cell suspension, and the cells were cultured and inoculated in 96-well cell culture plates. At 6 h, some cultured cells were taken out, added 20 μl MTT solution (5mg/ml), and cultured at 37°C for 4 h continuously. Absorbance of the supernatant containing impurities was carried out followed by addition of 150μl DMSO, and placing for 15min in a horizontal rocking. Optical density (OD) values at wavelength 480 nm were measured and the growth curves were plotted by enzyme-linked immunosorbent assay (ELISA) at 24, 48, 72 and 96 h.

Statistics

SPSS 19.6 (Beijing Strong Vinda Information Technology Co., Ltd., China) software system was used for statistical analyses; The basic enumeration data of patients was presented by [n (%)]; The measurement data was expressed in the form of mean±standard deviation;t-test was used for comparison between groups. P<0.05 had statistical significance.

Results

The expression of miR-140-5p and miR-370 in cancer tissues and adjacent tissues

The results of miR-140-5p and miR-370 detected by qRT-PCR showed that in cancer tissues the expression level of miR-140-5p was significantly lower than that in adjacent tissues, and the level of miR-370 was significantly higher than that in adjacent tissues, the difference been statistically significant (p<0.001) (Table 3).

Effect of miR-140-5p on nephroblastoma WT_CLS1 and SK-NEP-1 cells

The results after transfection of miR-140-5p to WT_CLS1 and SK-NEP-1 cells detected by MTT assay showed that the proliferative ability of WT_CLS1 and SK-NEP-1 cells in the two groups had no significant difference in 24 h (p>0.05). After 48 h, the proliferative ability of WT_CLS1 and SK-NEP-1 cells in miR-140-5p group was significantly lower than that in NLTF group with statistically significant difference (p<0.05) (Figure 1).

Effect of miR-370 on nephroblastoma WT_CLS1 and SK-NEP-1 cells

The results after transfection of miR-370 to WT_CLS1 and SK-NEP-1 cells detected by MTT assay showed that the proliferative ability of WT_CLS1 and SK-NEP-1 cells in the two groups had no significant difference in 24 h (p>0.05). After 48 h, the proliferative ability of WT_CLS1 and SK-NEP-1 cells in miR-370 group was significantly higher than that in NLTF group, the difference been statistically significant (p<0.05) (Figure 2).

Table 2. Primer sequence

Primer sequence	*F*	*R*
miR-140-5p	5'- CAGUGGUUUUACCCUAUGGUAG-3'	5'-ACCAUAGGGUAAAACCACUGUU-3'
mir-370	5'-GCCTGCTGGGGTGGAACCTGGTAA-3'	5'-GCGAGCACAGAATTAATACGAC-3'
U6	5'-UUCUCCGAACGUGU-CACGUTT-3	5'-ACGUGACACGUUCGGAGAATT-3'

Table 3. The expression of miR-140-5p and miR-370 in cancer tissues and adjacent tissues (n=60)

	Cancer tissues	*Adjacent tissues*	*t-test*	*p*
miR-140-5p	1.816±0.861	9.167±1.172	39.150	p<0.001
miR-370	8.127±1.147	2.186±1.115	28.770	p<0.001

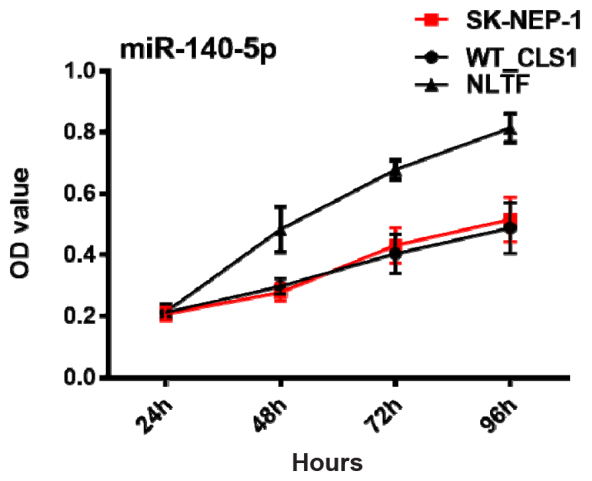

Figure 1. Effect of miR-140-5p on nephroblastoma WT_CLS1 and SK-NEP-1 cells. The proliferative ability of cells in miR-140-5p group and NLTF group had no significant difference in 24 h ($p>0.05$); After 48 h, the proliferative ability of cells in miR-140-5p group was significantly lower than that in NLTF group, and the difference was statistically significant ($p<0.05$).

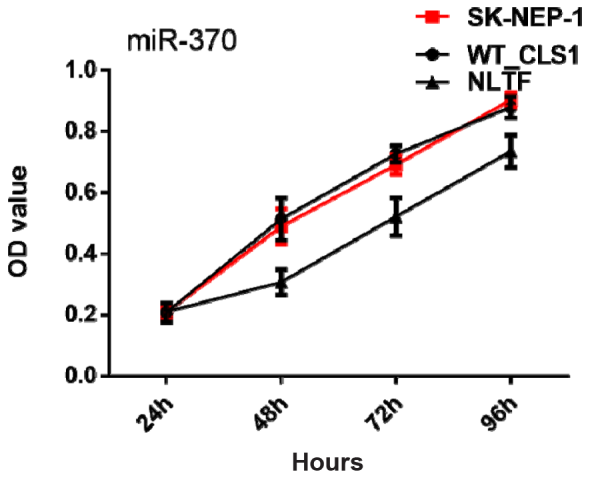

Figure 2. Effect of miR-370 on nephroblastoma WT_CLS1 and SK-NEP-1 cells. The proliferative ability of cells in miR-370 group and NLTF group had no significant difference in 24 h ($p>0.05$); After 48 h, the proliferative ability of cells in miR-370 group was significantly higher than that in NLTF group ($p<0.05$).

Discussion

Nephroblastoma is the most common type of renal malignancy in children, accounting for 6-7% of childhood malignancies. Most of the patients are children aged 0 to 5 years old, the average age of onset is 3.5 years, and the incidence rate is increasing gradually [15,16]. The clinical diagnosis of nephroblastoma is mainly based on abdominal ultrasonography, plain and enhanced CT scan. The accuracy of diagnosis is over 95% [17,18]. Nephroblastoma is sensitive to radiotherapy and chemotherapy, so most nephroblastoma patients can obtain good curative effect and prognosis after some active treatments such as operation, radiotherapy and triple chemotherapy [19]. MiRs, a class of non-coding small RNAs with 21-25 bases long and highly conserved can directly regulate the expression of genes and have a variety of important regulatory effects in cells [20]. It is reported that miRs regulate post-transcriptional genes and play an important role in tissue development and cell differentiation [21,22]. MiRs participate in a series of important processes in life. Therefore, by analyzing the expression of miR-140-5p and mir-370 in nephroblastoma and its proliferation in WT_CLS1 and SK-NEP-1 cells, researchers further clarified the role of these two miRs in nephroblastoma and provided reliable and effective reference for targeted gene therapy in this study.

In this experiment, miR-140-5 and miR-370 eukaryotic vector was constructed and transfected into nephroblastoma WT_CLS1 and SK-NEP-1 cells, and the expression changes and proliferation of both miRs in WT_CLS1 and SK-NEP-1 cells were detected. Compared with adjacent tissues, the expression of miR-140-5p was downregulated and the expression of miR-370 was upregulated in cancer tissues. MiR-140-5p was lowly expressed and miR-370 was highly expressed in nephroblastoma tissues. The results after transfection of miR-140-5p and miR-370 to WT_CLS1 and SK-NEP-1 cells detected by MTT assay showed that the proliferative ability of cells in miR-140-5p group was significantly lower than that in NLTF group, and the ability in miR-370 group was significantly higher than that in NLTF group, which indicated that miR-370 can effectively promote the proliferation of nephroblastoma cells. Studies by Su et al [23] showed that miR-140-5p was lowly expressed in cervical cancer cells and could significantly inhibit the proliferation and migration of cervical cancer cells, thus acting as an antioncogene, which was similar to our results. Researchers have reported that miR-370 promotes the invasion and metastasis of esophageal squamous cells and proliferation and metastasis of hepatocellular carcinoma cells by acting on several different target genes, which corroborates our study [24]. Our results suggest that miR-21 may be a potential marker for the diagnosis and risk prediction of colon cancer, while miR-138 can inhibit the proliferation of cancer cells and play a regulatory role.

Due to the limited experimental resources in the First Affiliated Hospital of Qiqihar Medical College, the experimental base was relatively

small. This study was only an *in vitro* experiment, and the mechanism of miR-140-5p and miR-370 nephroblastoma patients was not deeply studied and discussed. We will improve our experiments to achieve the best results in the future.

In conclusion, miR-140-5p is lowly expressed and miR-370 is highly expressed in nephroblastoma tissues. MiR-370 can promote the proliferation of nephroblastoma cells while miR-140-5p can inhibit their proliferation.

Conflict of interests

The authors declare no conflict of interests.

References

1. Kanyamuhunga A, Tuyisenge L, Stefan DC. Treating childhood cancer in Rwanda: the nephroblastoma example. Pan Afr Med J 2015; 21:326.
2. Ekenze SO, Agugua-Obianyo NE, Odetunde OA. The challenge of nephroblastoma in a developing country. Ann Oncol 2006;17:1598-1600.
3. Richards MK, Goldin AB, Savinkina A et al. The association between nephroblastoma-specific outcomes and high versus low volume treatment centers. J Pediatr Surg 2017;52:104-8.
4. Ito N. Experimental studies on tumors of the urinary system of rats induced by chemical carcinogens. Acta Pathol Jpn 1973;23:87-109.
5. Itoshima R, Kobayashi R, Sano H et al. Extrarenal Nephroblastoma of the Retroperitoneal Space in Children: A Case Report and Review of the Literature. J Pediatr Hematol Oncol 2017;39:296-8.
6. Dokmak S, Cabral C, Couvelard A, Aussilhou B, Belghiti J, Sauvanet A. Pancreatic metastasis from nephroblastoma: an unusual entity. JOP 2009; 10: 396-99.
7. Apoznański W, Sawicz-Birkowska K, Palczewski M, Szydełko T. Extrarenal nephroblastoma. Cent Eur J Urol 2015;68:153-6.
8. Zakaria OM, Daoud MY, Farrag SH, Mulhim AS. Efficacy of Different Protocols in Treatment of Nephroblastoma: A revisit. Gulf J Oncol 2016;1:55-60.
9. Stout TE, Au JK, Hicks JM, Gargollo PC. A Case of Bilateral Cystic Partially Differentiated Nephroblastoma vs Cystic Wilms' Tumor: Highlighting a Diagnostic Dilemma. Urology 2016;92:106-9.
10. Morel B, Jaudeau-Collart AC, Proisy M et al. Variability in Imaging Practices and Comparative Cumulative Effective Dose for Neuroblastoma and Nephroblastoma Patients at 6 Pediatric Oncology Centers. J Pediatr Hematol Oncol 2018;40:36-42.
11. Thomou T, Mori MA, Dreyfuss JM et al. Adipose-derived circulating miRNAs regulate gene expression in other tissues. Nature 2017;542:450-5.
12. Thind A,Wilson C. Exosomal miRNAs as cancer biomarkers and therapeutic targets. J Extracell Vesicles 2016;5:31292.
13. Yamane K, Naito H, Wakabayashi T et al. Regulation of SLD5 gene expression by miR-370 during acute growth of cancer cells. Sci Rep 2016;6:30941.
14. Lv J, Fan HX, Zhao XP et al. Long non-coding RNA Unigene56159 promotes epithelial-mesenchymal transition by acting as a ceRNA of miR-140-5p in hepatocellular carcinoma cells. Cancer Lett 2016;382:166-75.
15. Littooij AS, Sebire NJ, Olsen OE: Whole-tumor apparent diffusion coefficient measurements in nephroblastoma: Can it identify blastemal predominance? J Magn Reson Imaging 2017; 45: 1316-24.
16. Cafferata C, Cacciavillano W, Galluzzo ML, Flores P, Rose A, Zubizarreta P. Outcome of Nephroblastoma Treatment According to the SIOP-2001 Strategy at a Single Institution in Argentina. J Pediatr Hematol Oncol 2017;39:50-5.
17. Alatassi H, O'Bryan BE, Messer JC, Wang Z. Nephroblastoma Arising from Primary Testicular Germ Cell Tumor: A Case Report and Literature Review. Case Rep Pathol 2016;2016:7318672.
18. Tanaka N, Izawa T, Yamate J, Kuwamura M. Spontaneous nephroblastoma with striated muscle differentiation in an F344 rat. J Toxicol Pathol 2017;30:231-4.
19. Singh S, Gupta R, Khurana N. Cystic partially differentiated nephroblastoma: a rare differentiated variant of Wilm's tumour. J Postgrad Med 2006;52:45-6.
20. Venkatadri R, Muni T, Iyer AK, Yakisich JS, Azad N. Role of apoptosis-related miRNAs in resveratrol-induced breast cancer cell death. Cell Death Dis 2016; 7: e2104.
21. Madhavan D, Peng C, Wallwiener M et al. Circulating miRNAs with prognostic value in metastatic breast cancer and for early detection of metastasis. Carcinogenesis 2016;37:461-70.
22. Munagala R, Aqil F, Gupta RC. Exosomal miRNAs as biomarkers of recurrent lung cancer. Tumour Biol 2016;37:10703-14.
23. Su Y, Xiong J, Hu J, Wei X, Zhang X, Rao L. MicroRNA-140-5p targets insulin like growth factor 2 mRNA binding protein 1 (IGF2BP1) to suppress cervical cancer growth and metastasis. Oncotarget 2016;7:68397-411.
24. Pan X, Wang H, Tong D et al. Physcion induces apoptosis in hepatocellular carcinoma by modulating miR-370. Am J Cancer Res 2016;6:2919-31.