Med Oncol (2013) 30:353
DOI 10.1007/s12032-012-0353-2

ORIGINAL PAPER

Prognostic significance of serum miR-17-5p in lung cancer

Qun Chen · Qing Si · Song Xiao · Qiang Xie · Jiangping Lin · Chenhui Wang · Lizhou Chen · Qiaolin Chen · Lin Wang

Received: 29 July 2012 / Accepted: 7 August 2012 / Published online: 24 December 2012

Abstract miR-17-5p is abnormally expressed in various tumor types. The aim of this study was to investigate the expression level of miR-17-5p in serum of patients with lung cancer and to determine whether serum miR-17-5p expression is related to the prognosis of patients with lung cancer. RT-qPCR was used to examine expression of miRNA-17-5p in 20 pairs of lung cancer and adjacent normal tissues, and sera from 221 patients with lung cancer and 54 matched controls. The correlation of serum miR-17-5p with clinicopathological factors or prognosis of patients with lung cancer was analyzed. The expression level of miR-17-5p obviously increased in lung cancer tissues ($P = 0.004$). Furthermore, serum miR-17-5p expression also significantly increased in patients with lung cancer compared with healthy individuals ($P = 0.03$). The survival analysis showed that serum miR-17-5p expression was closely related to the survival of patients with lung cancer. Patients with high miR-17-5p expression had shorter survival times [hazard ratio (HR) = 1.767, 95 %CI 1.039-3.005, $P = 0.035$]. A lower expression level of serum miR-17-5p helps extend the survival of patients with lung cancer. Thus, miR-17-5p may be potential biomarker for prediction the prognosis in patients with lung cancer.

Keywords miR-17-5p · Lung cancer · MicroRNA · Prognosis · Serum · Survival · miR-17-92

Q. Chen (✉) · Q. Si · S. Xiao · Q. Xie · J. Lin · C. Wang · L. Chen · Q. Chen · L. Wang
Department of Oncology, Fuzhou Pulmonary Hospital, Fujian Medical University, Fuzhou 350008, China
e-mail: chenqun88@hotmail.com

Introduction

Lung cancer remains one of the major cancer diseases worldwide. Approximately 1.3 million patients with lung cancer die each year, making it the cancer with the highest death toll [1]. Most patients with lung cancer will progress and ultimately die of metastasis cancer. Recent study has shown that the average five-year survival rate of patients with lung cancer is only 17.1 % [2]. A variety of screening methods have been used to predict the prognosis of patients with lung cancer, but these methods, including imaging and cytology studies, failed to effectively improve patient survival [3, 4].

MicroRNAs are endogenous non-coding small RNA molecules that are extensively being studied at present. Their lengths range from 19 to 29 nucleotides. MicroRNAs are involved in regulating the various pathways in eukaryotes. Abnormal expression of microRNAs has been found in a variety of malignant cells, which is closely related to activation of oncogenes and inactivation of anti-oncogenes [5–7]. Rothschild et al. [8] found that increased miR-381 expression in lung cancer cells inhibited the expression of ID1 gene and significantly decreased cell migration and invasion. Low expression of miR-381 was associated with poor prognosis of lung cancer. Gaku et al. [9] reported that miR-542-5p can inhibit the expression of EGFR gene in lung cancer cell line H325, thereby inhibiting cancer cell growth. Therefore, microRNAs are closely related to cancer development, which can be used as potential molecular markers for cancers [10–12] and may be used as new forms of cancer treatment [13].

The miR-17-5p originates from the mature microRNA gene product of the miR-17 gene precursor 5p. Aberrant expression of the miR-17-5p is observed in a variety of cancers, including lung cancer [14–16], pancreatic cancer

[17], liver cancer [18, 19], breast cancer [20, 21], leukemia [22], gastric cancer [23] and cervical cancer [24]. The miR-17-5p is markedly overexpressed in lung cancers [14]. Reduced expression of the miR-17-5p inhibited the growth and promoted the apoptosis in A549 cells [15]. Furthermore, the expression levels of the miR-17-5p gene were significantly increased in hepatocellular carcinoma (HCC) tissues and in serum of patients with gastric cancer, and miR-17-5p overexpression was correlated with poor prognosis [19, 23]. These previous findings suggest that miR-17-5p has important roles in the occurrence and development of many types of cancer. Therefore, in the present study, we investigated the expression of the miR-17-5p in lung cancer tissues and in serum from 221 patients with lung cancer and 54 healthy controls, and determined whether serum miR-17-5p expression was related to the prognosis of lung cancer.

Materials and methods

Subjects

Tissue and serum samples used in this study were collected from Fuzhou Pulmonary Hospital. A total of 20 paired of lung cancer and adjacent normal tissues were collected from patients who received surgery in Fuzhou Pulmonary Hospital from March 2011 to November 2011. In addition, the serum of 221 patients with lung cancer and the serum of 54 healthy volunteers who have the same age and gender distributions were collected. All patients were diagnosed with lung cancer for the first time and have never received chemotherapy, radiotherapy or other tumor-specific treatment. All patients with lung cancer were pathologically diagnosed with lung cancer. Patients known to have a family history of cancer or metastasized cancer from other or unknown origins were excluded. All controls had no family history of cancer and no past history of cancer. All controls were non-smokers. The study was approved by the ethics committee of Fuzhou Pulmonary Hospital. Collection of samples was performed after obtaining consent from the patients and control subjects.

RNA purification

The total RNA was extracted from tissue using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The mirVana^{TM} miRNA Isolation Kit (Ambion, Austin, TX, USA) was used to purify RNA from serum samples according to the manufacture's instructions. RNA was quantified spectrophotometrically with the NanoDrop ND-1000 (NanoDrop, Wilmington, DE) and stored at −80 °C.

RT-PCR and quantitative RT-PCR (qRT-PCR)

The reverse transcription was performed using the One Step PrimeScript® microRNA cDNA Synthesis Kit (Takara, Dalian, China) according to the manufacturer's instructions. The reverse transcription reaction system contained 2 × microRNA Reaction Buffer Mix (10 μl), 0.1 % bovine serum albumin (BSA, 2 μl), 1U microRNA PrimeScript® RT Enzyme Mix (2 μl), total RNA extract (1 μl) and RNase-free ddH_2O (5 μl). The reverse transcription reaction mixture was incubated at 37 °C for 60 min, followed by denaturation at 85 °C for 5 s.

The qRT-PCR was performed following the instructions on the SYBR® Premix Ex Taq^{TM} II Kit (Takara, Dalian, China). Each qRT-PCR reaction mixture contained the following: 12.5 μl SYBR® Premix Ex Taq^{TM} II, 1 μl PCR forward primer, 1 μl uni-miR qPCR primer, 2 μl cDNA from the RT-PCR and 8.5 μl ddH_2O. The following reaction conditions were used for qRT-PCR: initial denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 5 s and 60 °C for 30 s. The qPCR reactions were performed on an ABI7500 Fast Real-time PCR system (Applied Biosystems, CA, USA), with three parallel replicates for each sample. U6 snRNA was used as the gene reference to normalize microRNA qRT-PCR data.

Statistical analysis

The relative miR-17-5p expression level for each subject was calculated using the formula $2^{-\Delta\Delta Ct}$ [25]. The paired *t* test was used to compare the differential expression in lung cancer and normal counterpart tissues, as well as in the serum of patients with lung cancer and normal healthy individuals. The unilateral ANOVA test was used to analyze the relationship between miR-17-5p expression and the clinical pathological features of the patients. The Kaplan–Meier method was used to estimate the cumulative probability of survival, and statistical significance was assessed using the log-rank test. Univariate and multivariate statistical analyses were carried out using Cox proportional hazards regression model to investigate the effects of patients' characteristics (miR-17-5p expression, smoking status, gender, age, TNM, histology) on overall survival (OS). A value of $P < 0.05$ was regarded as statistically significant. Statistical analysis was performed using SPSS 17.0 (SPSS Inc, Chicago, USA).

Results

miR-17-5p overexpression in lung cancer tissues and serum

The qRT-PCR was performed to analyze the expression of the miR-17-5p in 20 pairs of lung cancer and their adjacent tissues. The expression level of miR-17-5p in adjacent normal tissues was standardized to a value of 1. The expression levels of miR-17-5p in lung cancer tissues were analyzed through a homogenized analysis compared with their adjacent tissues. The expression of miR-17-5p in lung cancer tissue was significantly higher than that in the adjacent normal tissue ($P = 0.004$) (Fig. 1a). In addition, serum miR-17-5p expression was also analyzed in the serum of 221 cancer patients and 54 healthy volunteers. The expression levels of serum miR-17-5p were significantly elevated in patients with lung cancer ($P = 0.03$) (Figure 1b).

The correlation between serum miR-17-5p expression and pathological features

The relationship between serum miR-17-5p expression and clinical pathological features was evaluated in order to better understand the potential role of miR-17-5p in the development and progression of lung cancer. Unilateral ANOVA analysis was used to determine the relationship between the miR-17-5p expression and the clinical characteristics, including age, sex, histology, TNM and smoking status. Serum miR-17-5p expression was significantly related to the smoking status and TNM ($P = 0.005$ and $P = 0.031$, respectively) (Table 1).

Table 1 Characteristic of patients with lung cancer serum

Characteristic	miR7-5p		*P* value
	High expression	Low expression	
Median (range)			
221 cancer serum	2.37 ± 1.97		
54 control serum	1.08 ± 1.98		
No.	101	120	
Age (years)			0.134
<60	53	75	
≥60	48	45	
Gender			0.920
Male	73	86	
Female	28	34	
Smoking status			0.005
Never	29	61	
Current	4	0	
Former	44	56	
Unknown	24	2	
TNM			0.031
I	35	26	
II	30	44	
III	34	39	
IV	2	11	
Histology			0.060
Squamous cell carcinoma	46	38	
Adenocarcinoma	32	55	
Adenosquamous	14	23	
Others	9	4	

The correlation between serum miR-17-5p expression and survival in lung cancer

Serum miR-17-5p expression was significantly related to the survival of patients with lung cancer. Patients with high miR-17-5p expression had a median survival time (MST) of 33 months, whereas patients with low miR-17-5p expression had an MST of 40 months (log-rank test $P = 0.0296$) (Fig. 2a). In the Cox proportional hazard model, we found that patients with high miR-17-5p expression had shorter OS than those with lower miR-17-5p expression after adjusted for age, sex, TNM, smoking status and histology [hazard ratio (HR) = 1.767, 95 %CI 1.039-3.005, $P = 0.035$] (Table 2). The survival was poor especially for patients with high miR-17-5p expression and TNM stages of III–IV or a past history of cigarette smoking

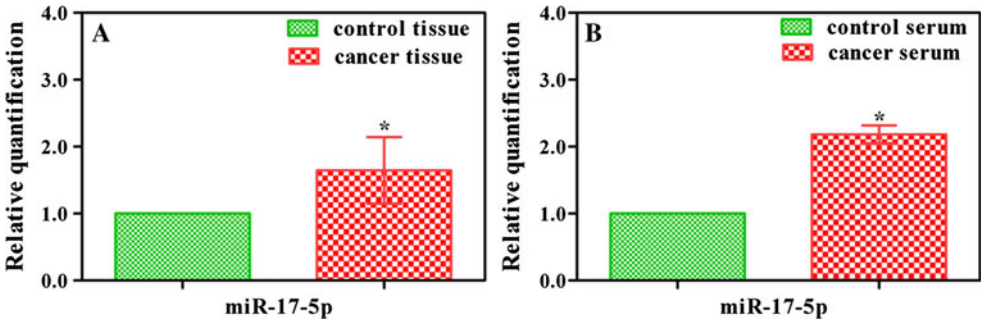

Fig. 1 miR-17-5p expression in lung tissues and serum. **a** Expression difference of miR-17-5p in lung cancer and adjacent tissue. **b** Serum miR-17-5p expression. *$P < 0.05$

(TNM III–IV: HR = 2.403, 95 % CI 0.57–7.322; former smoker: HR = 6.818, 95 % CI 2.131–21.811) (Fig. 2b, c).

Discussion

Increasing numbers of studies have shown that microRNA can act either as tumor proto-oncogenes or tumor suppressor gene [5, 7]. Deregulated microRNA expression is closely related to the occurrence, development and prognosis of a variety of malignant tumors [5, 10, 26–31]. The miR-17-92 gene cluster is a highly conserved gene cluster that encodes miR-17-5p, miR-17-3p, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92-1 [32]. In most of previous studies, the expression of the miR-17-92 gene cluster is increased in multiple tumor types, such as colorectal cancer [33], lung cancer [14], pancreatic cancer [17] and HCC [19]. The miR-17-92 cluster plays important roles in coordinating many cellular processes, particularly those involved in the occurrence and development of cancer [33, 34].

In the present study, miR-17-5p was identified to be upregulated in lung cancer tissues compared with in adjacent normal tissues. This result is consistent with that of previous study [14, 16]. By constructing a network model, Cloonan et al. [35] found that miR-17-5P could be regarded as both an oncogene and a tumor suppressor gene in different cell environments, depending on the expression of other transcriptional regulators. The high expression of miR-17-5p can promote lung cancer cell growth and inhibit the apoptosis of lung cancer cells [15]. Wang et al. [23] reported that the miR-17-5p expression is increased in gastric cancer, and its serum expression level is negatively correlated with survival time. Chen et al. [19] reported that miR-17-5p expression is increased in HCC tissues, and patients with high miR-17-5p expression have poor prognosis [19]. Li et al. [20] found that miR-17-5p could promote the invasion and metastasis of breast cancer cells by inhibiting the HMG box-containing protein 1 (HBP1) expression. However, Hossain et al. [21] found that miR17-5p expression was decreased in breast cancer cell lines, and miR17-5p could inhibit cancer cell proliferation by inhibiting the translation of AIB1 and E2F1. These studies suggest that the functional mechanism of the miR-17-5p in cancer is highly complex, and further research is needed.

Table 2 Univariate Cox proportional hazard analysis of cancer characteristic for survival rate

Features	Subset	Hazard ratio (95 % CI)	*P* value
Age (years)	<60/≥60	1.073 (0.654–1.763)	0.780
Gender	Male/female	0.846 (0.496–1.442)	0.539
Smoking status	Never/current/former	1.160 (0.644–2.089)	0.621
TNM	I/II/III/IV	0.636 (0.410–0.987	0.044
Histology	Adenocarcinoma/ adenosquamous/ SCC/other	1.155 (0.890–1.500)	0.278
miR-17-5p	High expression/ low expression	1.767 (1.039–3.005)	0.035

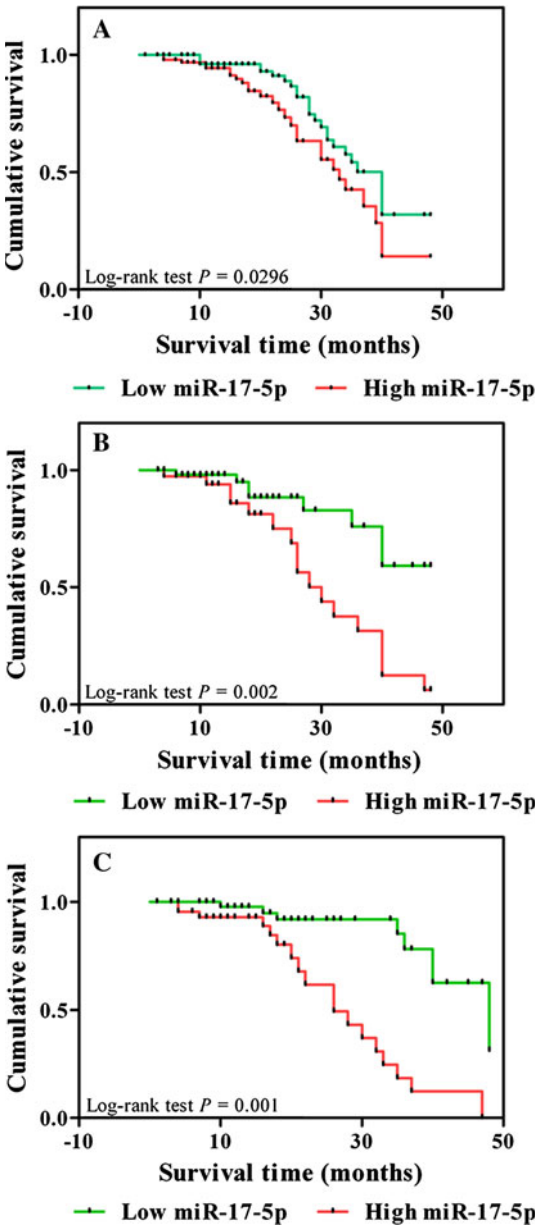

Fig. 2 Kaplan–Meier survival curves for patients with lung cancer plotted on serum miR-17-5p expression. **a** All patients with lung cancer. **b** Patients with stage III-IV. **c** Ever-smoking patients

The microRNAs in tumor cells can enter the blood circulation, and the expression level of serum microRNA can reflect its expression level in tissues to some extent. Therefore, circulation microRNAs can be used as biomarkers for tumor detection [7, 12]. In the represent study, the expression levels of the miR-17-5p in lung cancer serum were significantly increased compared with those in normal control serum. Heegaard er al. [36] reported that serum miR-17-5p was decreased in patients with early stage non-small cell lung cancer. However, this phenomenon was not observed in the present study. Previous studies have shown that the miR-17-5p acts as both an oncogene and a tumor suppressor in breast cancer cell lines [20, 21]. Furthermore, miR-17-5p overexpression was found in high-invasive MDA-MB-231 breast cancer cells but not in low-invasive MCF-7 breast cancer cells. In this study, we found that patients with miR-17-5p overexpression had worse survival. This effect was more pronounced in patients with stage III/IV than those with stage I/II ($P = 0.002$). This result is in accordance with oncogenic properties of the miR-17-5p. Therefore, the miR-17-5p may play different roles at different stages of lung cancer, which might explain, to some extent, the discrepant results. Further studies are required to elucidate the underlying mechanisms of the miR-17-5p in tumorigenesis.

In conclusion, the findings of this study indicated that miR-17-5p overexpression in serum was correlated with worse survival. Serum miR-17-5p expression may be an independent prognostic factor for patients with lung cancer. However, due to the small sample size in this study, these results have yet to be verified by large-scale studies.

Acknowledgments This work was supported by grant from the Wu Jieping Medical Foundation (320.6250.1272).

Conflict of interest None.

References

1. Papadimitrakopoulou V. Development of PI3 K/AKT/mTOR Pathway Inhibitors and Their Application in Personalized Therapy for Non-Small-Cell Lung Cancer. J Thorac Oncol. 2012;7(8).
2. Siegel R, Desantis C, Virgo K, Stein K, Mariotto A, Smith T, et al. Cancer treatment and survivorship statistics. CA Cancer J Clin. 2012;62(4):220–41.
3. Manser RL, Irving LB, Stone C, Byrnes G, Abramson M, Campbell D. Screening for lung cancer. Cochrane Database Syst Rev. 2004;1 CD001991.
4. Bach PB, Jett JR, Pastorino U, Tockman MS, Swensen SJ, Begg CB. Computed tomography screening and lung cancer outcomes. JAMA. 2007;297(9):953–61.
5. Cho WC. OncomiRs: the discovery and progress of microRNAs in cancers. Mol Cancer. 2007;6:60.
6. Kong YW, Ferland-McCollough D, Jackson TJ, Bushell M. microRNAs in cancer management. Lancet Oncol. 2012;13(6): e249–58.
7. Lages E, Ipas H, Guttin A, Nesr H, Berger F, Issartel JP. MicroRNAs: molecular features and role in cancer. Front Biosci. 2012;17:2508–40.
8. Rothschild SI, Tschan MP, Jaggi R, Fey MF, Gugger M, Gautschi O. MicroRNA-381 Represses ID1 and is Deregulated in Lung Adenocarcinoma. J Thorac Oncol. 2012;7(7):1069–77.
9. Yamaguchi G, Takanashi M, Tanaka M, Fujita K, Ohira T, Kuroda M, et al. Isolation of miRNAs that target EGFR mRNA in human lung cancer. Biochem Biophys Res Commun. 2012; 420(2):411–6.
10. Liu R, Chen X, Du Y, Yao W, Shen L, Wang C, et al. Serum microRNA expression profile as a biomarker in the diagnosis and prognosis of pancreatic cancer. Clin Chem. 2012;58(3):610–8.
11. Kuner R, Brase JC, Sultmann H, Wuttig D. microRNA biomarkers in body fluids of prostate cancer patients. Methods. 2012.
12. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. 2008;105(30):10513–8.
13. Kota J, Chivukula RR, O'Donnell KA, Wentzel EA, Montgomery CL, Hwang HW, et al. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. Cell. 2009;137(6):1005–17.
14. Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, et al. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res. 2005;65(21):9628–32.
15. Matsubara H, Takeuchi T, Nishikawa E, Yanagisawa K, Hayashita Y, Ebi H, et al. Apoptosis induction by antisense oligonucleotides against miR-17-5p and miR-20a in lung cancers overexpressing miR-17-92. Oncogene. 2007;26(41):6099–105.
16. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A. 2006; 103(7):2257–61.
17. Yu J, Ohuchida K, Mizumoto K, Fujita H, Nakata K, Tanaka M. MicroRNA miR-17-5p is overexpressed in pancreatic cancer, associated with a poor prognosis, and involved in cancer cell proliferation and invasion. Cancer Biol Ther. 2010;10(8):748–57.
18. Yang F, Yin Y, Wang F, Wang Y, Zhang L, Tang Y, et al. miR-17-5p Promotes migration of human hepatocellular carcinoma cells through the p38 mitogen-activated protein kinase-heat shock protein 27 pathway. Hepatology. 2010;51(5):1614–23.
19. Chen L, Jiang M, Yuan W, Tang H. miR-17-5p as a novel prognostic marker for hepatocellular carcinoma. J Invest Surg. 2012;25(3):156–61.
20. Li H, Bian C, Liao L, Li J, Zhao RC. miR-17-5p promotes human breast cancer cell migration and invasion through suppression of HBP1. Breast Cancer Res Treat. 2011;126(3):565–75.
21. Hossain A, Kuo MT, Saunders GF. Mir-17-5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA. Mol Cell Biol. 2006;26(21):8191–201.
22. Mraz M, Pospisilova S, Malinova K, Slapak I, Mayer J. MicroRNAs in chronic lymphocytic leukemia pathogenesis and disease subtypes. Leuk Lymphoma. 2009;50(3):506–9.
23. Wang M, Gu H, Wang S, Qian H, Zhu W, Zhang L, et al. Circulating miR-17-5p and miR-20a: molecular markers for gastric cancer. Mol Med Rep. 2012;5(6):1514–20.
24. Wei Q, Li YX, Liu M, Li X, Tang H. MiR-17-5p targets TP53INP1 and regulates cell proliferation and apoptosis of cervical cancer cells. IUBMB Life. 2012;64(8):697–704.
25. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402–8.
26. Liu C, Kelnar K, Vlassov AV, Brown D, Wang J, Tang DG. Distinct microRNA Expression Profiles in Prostate Cancer Stem/

Progenitor Cells and Tumor-Suppressive Functions of let-7. Cancer Res. 2012;72(13):3393–404.
27. Png KJ, Halberg N, Yoshida M, Tavazoie SF. A microRNA regulon that mediates endothelial recruitment and metastasis by cancer cells. Nature. 2012;481(7380):190–4.
28. Lujambio A, Lowe SW. The microcosmos of cancer. Nature. 2012;482(7385):347–55.
29. Benhamed M, Herbig U, Ye T, Dejean A, Bischof O. Senescence is an endogenous trigger for microRNA-directed transcriptional gene silencing in human cells. Nat Cell Biol. 2012;14(3):266–75.
30. Aceto N, Sausgruber N, Brinkhaus H, Gaidatzis D, Martiny-Baron G, Mazzarol G, et al. Tyrosine phosphatase SHP2 promotes breast cancer progression and maintains tumor-initiating cells via activation of key transcription factors and a positive feedback signaling loop. Nat Med. 2012;18(4):529–37.
31. Rottiers V, Naar AM. MicroRNAs in metabolism and metabolic disorders. Nat Rev Mol Cell Biol. 2012;13(4):239–50.
32. Aguda BD, Kim Y, Piper-Hunter MG, Friedman A, Marsh CB. MicroRNA regulation of a cancer network: consequences of the feedback loops involving miR-17-92, E2F, and Myc. Proc Natl Acad Sci U S A. 2008;105(50):19678–83.
33. Diosdado B, van de Wiel MA. Terhaar Sive Droste JS, Mongera S, Postma C, Meijerink WJ et al. MiR-17-92 cluster is associated with 13q gain and c-myc expression during colorectal adenoma to adenocarcinoma progression. Br J Cancer. 2009;101(4):707–14.
34. Mendell JT. miRiad roles for the miR-17-92 cluster in development and disease. Cell. 2008;133(2):217–22.
35. Cloonan N, Brown MK, Steptoe AL, Wani S, Chan WL, Forrest AR, et al. The miR-17-5p microRNA is a key regulator of the G1/S phase cell cycle transition. Genome Biol. 2008;9(8):R127.
36. Heegaard NH, Schetter AJ, Welsh JA, Yoneda M, Bowman ED, Harris CC. Circulating micro-RNA expression profiles in early stage nonsmall cell lung cancer. Int J Cancer. 2012;130(6):1378–86.