Int J Clin Exp Pathol 2017;10(3):3409-3416
www.ijcep.com /ISSN:1936-2625/IJCEP0036244

Original Article

Circulating miR-17-5p as a potential biomarker for diagnosis and prognosis in osteosarcoma

Hongxuan Zhu[1], Qiande Liao[1], Ke Yin[2], Da Zhong[1], Chenggong Wang[1]

[1]Department of Orthopedics, Xiangya Hospital, Central South University, Changsha, Hunan, China; [2]Department of Orthopedics, First Affiliated Hospital of South China University, Hengyang, Hunan, China

Received July 16, 2016; Accepted December 21, 2016; Epub March 1, 2017; Published March 15, 2017

Abstract: The study was designed to explore the usefulness of miR-17-5p as a biomarker for the diagnosis and prognosis of osteosarcoma. Circulating miR-17-5p expression levels were determined by qRT-PCR in 62 osteosarcoma patients and 36 healthy volunteers. It was found that expression of miR-17-5p was significantly elevated in patients with osteosarcoma compared with the control group ($P < 0.001$). ROC analysis comparison of relative miR-17-5p expression levels in osteosarcoma patients and healthy controls, using a cut-off value of 0.88, yielded an area under the ROC curve of 0.793 ($P < 0.001$; 95% confidence interval, 0.700-0.868), with sensitivity and specificity of 50.0% and 97.2%, respectively. High miR-17-5p expression was significantly associated with advanced tumor stage ($P < 0.001$). Kaplan-Meier, and univariate and multivariate Cox regression analyses all demonstrated that patients with high miR-17-5p expression exhibited shorter progression-free ($P < 0.001$, $P < 0.001$, and $P=0.001$, respectively) and overall ($P < 0.001$, $P < 0.001$, and $P=0.002$, respectively) survival. These finding warrants that overexpression of circulating miR-17-5p has potential as a novel diagnostic and prognostic biomarker in osteosarcoma.

Keywords: Osteosarcoma, miR-17-5p, diagnosis, prognosis

Introduction

Osteosarcoma (OS) generally localizes to the metaphysis of long bones and is the most common bone tumor in children and adolescents, comprising 2.4% of all malignancies in pediatric patients [1, 2]. It is also a major cause of malignancy-related death in adolescence, due to its rapid progression and high metastatic potential [2, 3]. At present, the main treatment strategies for OS consist of tumor excision, chemotherapy, and radiotherapy, and the five-year survival rate of OS patients is approximately 60%-70% [4, 5]. However, a large proportion of OS patients respond poorly to combined therapy and their risk of local relapse and distant metastasis is relatively high [6]. The unfavorable prognosis of OS patients is partially due to current poor strategies for early diagnosis and evaluation of prognosis [7]. Therefore, there is an urgent need to identify novel diagnostic and prognostic biomarkers to improve the clinical outcome of OS.

MicroRNAs are a group of single-stranded, endogenous and non-coding RNAs that are generally 18-24 nucleotides in length [8]. These molecules can modulate gene expression by binding to the 3'-untranslated regions (3'-UTR) of mRNAs [9, 10] and they participate in various biological and pathological processes, including development, apoptosis, cell differentiation, cell proliferation, and inflammation [11-13]. Dysregulation of microRNAs, including miR-421, miR-133b, miR-503, miR-191, miR-130, and miR-125b (among others), has previously been reported in OS [14-17]. miR-17-5p is a member of the miR-17-92 microRNA cluster, and is implicated in multiple malignant tumors, including hepatocellular carcinoma, Ewing's sarcoma, ovarian cancer, and breast cancer [8, 10, 18-20]. Of relevance to OS, miR-17-5p is overexpressed in OS cell lines and tissues, and up-regulation of miR-17-5p correlates with advanced TNM stage and tumor growth [8]. Further investigation of the role of miR-17-5p in OS is needed, and evidence from clinical practice is also in urgently required.

In the present study, we explored the role of miR-17-5p in the diagnosis and assessment of prognosis of OS.

Table 1. Pathological and clinical parameters of enrolled subjects

Characteristics	Osteosarcoma (n=62)	Control (n=36)	P
Age (years)	23.3±10.2	22.3±7.3	0.585
Gender			
Male	40	18	0.202
Female	22	18	
Tumor location			
Tibia/femur	48		
Elsewhere	14		
Tumor stage			
T1	34		
T2	22		
T3	6		
Histologic grade			
G1	18		
G2	19		
G3	25		
miR-17-5p in plasma	0.97±0.37	0.63±0.17	< 0.001

Patients and methods

Patients

The study was approved by the Ethical Committee of Xiangya Hospital, and informed consent was obtained from all enrolled participants. From January 2008 to December 2012, primary OS patients administered in our department were screened for eligibility for inclusion in the study. Patients meeting the following criteria were excluded: metastatic OS, previous malignant tumor in other organs or systems, history of cardiovascular or cerebral vascular disease, poor general physical condition, previous chemotherapy, radiotherapy or surgery, and unwillingness to participate. All enrolled patients received neuadjuvant chemotherapy, tumor resection, and adjuvant therapy according to NCCN (National Comprehensive Cancer Network) clinical practice guidelines on OS, and patients were followed-up via outpatient visit, telephone, and letter. Patients were asked to undergo physical examination and radiography every 3 months, and computed tomography or magnetic resonance imaging when necessary. Progression-free survival was calculated from the date of surgery to recurrence or metastasis, and overall survival was calculated from the date of surgery to that of death. An age- and gender-matched healthy control group was also recruited.

Samples and quantitative real-time PCR (qRT-PCR)

Peripheral venous blood samples (5 ml) were collected into EDTA anticoagulation tubes, before any treatment was administered, and centrifuged at 4,000×*g* for 15 min to separate plasma within 20 min of collection. Plasma samples were transferred into clean micro-centrifuge tubes and centrifuged again at 12,000×*g* for 5 min. Supernatant plasma samples (200 μl) were collected and stored at -80°C until analysis.

A High Pure miRNA Isolation kit (Roche, Mannheim, Germany) was used to isolate miRNA, according to the manufacturer's instructions. RNA concentration was quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

A RevertAid First Strand cDNA synthesis kit (Fermentas, Burlington, Canada) was used to perform reverse transcription reactions. Reactions (total volume, 10 μl) consisted of random primers (0.5 μl), RNase inhibitor (0.5 μl), multiscribe reverse transcriptase (0.5 μl), dNTPs (1 μl, 10 mmol/L), 5x reverse transcription buffer (2 μl), RNA sample (100 ng), and RNase-free water, and were incubated at 65°C for 5 min, followed by incubation at 42°C for 60 min, 70°C for 5 min, and a final hold step at 4°C.

A Rotor gene 6000 real-time PCR machine (Qiagen, Germany) was used to conduct qPCR, and 20 μl qPCR reactions comprised TaqMan 2X Perfect Master Mix (10 μl), cDNA solution (1 μl), forward and reverse primers (0.5 μl each), and RNase-free water (8 μl). U6 snRNA (Ambion, AM30303) was used as an internal control. The primers used for qPCR were: miR-17-5p forward, 5'-GCCGCAAAGTGCTTACAGTG-3' and reverse, 5'-TGCAGGGTCCGAGGTAT-3'; and U6 forward, 5'-CTCGCTTCGGCAGCACATATACT-3', and reverse, 5'-ACGCTTCACGAATTTGCGTG-TC-3'. PCR amplification cycles were as follows: an initial denaturation at 95°C for 10 min, followed by 35 cycles of 95°C for 30 s, and 60°C for 1 min; reactions were terminated by incuba-

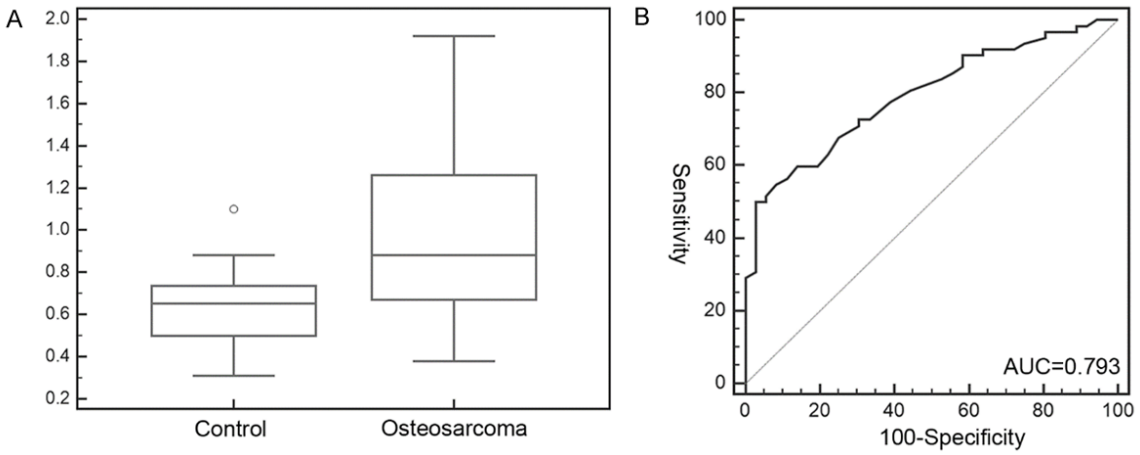

Figure 1. Circulating miR-17-5p is up-regulated in osteosarcoma. A. Comparison of miR-17-5p expression levels in osteosarcoma and control groups. B. Receiver-operating characteristics curve analysis of the use of plasma miR-17-5p for the detection of osteosarcoma.

Table 2. Comparison of miR-17-5p expression levels stratified for various patient characteristics

Characteristics	N	miR-17-5p expression	P
Age (years)			0.849
< 25	42	0.97±0.36	
≥25	20	0.99±0.41	
Gender			0.054
Male	40	1.04±0.39	
Female	22	0.85±0.31	
Tumor location			0.588
Tibia/femur	48	0.99±0.38	
Elsewhere	14	0.93±0.41	
Tumor stage			< 0.001
T1	34	0.80±0.23	
T2	22	1.09±0.40	
T3	6	1.56±0.18	
Histologic grade			0.405
G1		0.99±0.45	
G2		0.88±0.35	
G3		1.03±0.33	

tion at 95°C for 15 s and then held at 4°C. The relative expression of miR-17-5p was calculated using the comparative C_T (ΔΔCT) method, and each reaction was performed in triplicate.

Statistical analyses

MedCalc 13.0 (MedCalc Software bvba, Ostend, Belgium) and SPSS 16.0 (SPSS, Chicago, IL, USA) were used for statistical analyses. One way analysis of variance and Student's t tests were used for evaluation of differences between groups, as appropriate. Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the efficacy of miR-17-5p for the differential diagnosis of OS and healthy controls. The association between miR-17-5p expression and survival was assessed by Log-rank test and Cox proportional hazard regression analysis. Age, gender, tumor location, tumor stage, histologic grade, and miR-17-5p expression level were included in the multivariate analysis. The mean value of miR-17-5p expression in the OS group was set as the cut-off point for differentiation of patients with high or low miR-17-5p expression. $P < 0.05$ was considered statistically significant.

Results

A total of 62 OS patients and 36 healthy volunteers were enrolled in the study. There were no significant differences, in terms of age and gender, between the two groups (P=0.585 and P=0.202, respectively). The characteristics of enrolled subjects are presented in **Table 1**.

The expression level of miR-17-5p in the OS group was significantly elevated compared with that in the control group ($P < 0.001$; **Figure 1A**). ROC analysis, using the cut-off point of a relative expression level of 0.88, demonstrated that miR-17-5p yielded an area under the ROC

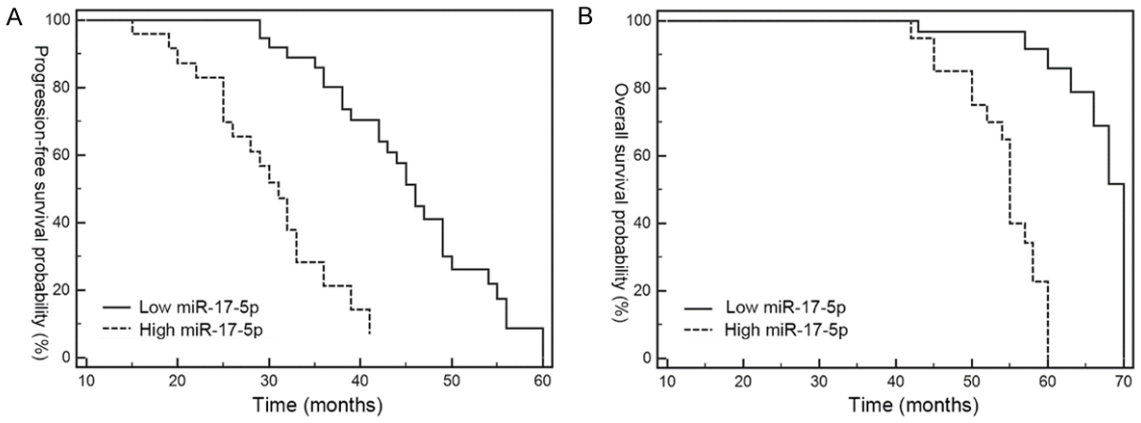

Figure 2. Kaplan-Meier analysis of the association between miR-17-5p expression and (A) progression-free survival, and (B) overall survival of osteosarcoma patients.

Table 3. Cox proportional regression analysis of the association between miR-17-5p expression levels in plasma and progression-free survival of osteosarcoma patients

Covariate	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	P
Age (years)			0.788			0.409
< 25	1.000			1.000		
≥25	0.920	0.500-1.691		0.757	0.391-1.466	
Gender			0.270			0.910
Female	1.000			1.000		
Male	1.419	0.762-2.646		0.960	0.474-1.945	
Tumor location			0.196			0.392
Tibia/femur	1.000			1.000		
Elsewhere	1.557	0.795-3.050		1.400	0.648-3.022	
Tumor stage			< 0.001			0.009
T1	1.000			1.000		
T2+T3	6.038	2.834-12.866		3.346	1.345-8.329	
Histologic grade			0.839			0.713
G1	1.000			1.000		
G2+G3	1.065	0.581-1.952		0.886	0.464-1.691	
miR-17-5p expression			< 0.001			0.001
Low	1.000			1.000		
High	7.116	3.208-15.786		5.526	2.015-15.155	

curve (AUC) of 0.793 (P < 0.001; 95% confidence interval (CI), 0.700-0.868), in distinguishing OS patients from healthy controls. The sensitivity and specificity of the discrimination between the two groups were 50.0% and 97.2%, respectively (**Figure 1B**). There was no significant association between miR-17-5p expression and age, gender, tumor location, or histologic grade, while high miR-17-5p expression was significantly associated with advanced tumor stage (P < 0.001; **Table 2**).

In survival analysis, patients with miR-17-5p levels < 0.97 were assigned to the low miR-17-5p expression group, while the remaining patients were classified as having high miR-17-5p expression. Kaplan-Meier analysis demonstrated that patients with high miR-17-5p levels suffered shorter progression-free survival (P < 0.001; **Figure 2A**), and this finding was verified by univariate and multivariate Cox regression analysis (P < 0.001 and P=0.001, respectively; **Table 3**). Univariate and multivariate Cox regres-

Table 4. Cox proportional regression analysis of the association between miR-17-5p expression in plasma and overall survival of osteosarcoma patients

Covariate	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	P
Age (years)			0.716			0.745
< 25	1.000			1.000		
≥25	0.858	0.376-1.958		0.861	0.348-2.129	
Gender			0.152			0.702
Female	1.000			1.000		
Male	1.821	0.802-4.136		0.814	0.284-2.335	
Tumor location			0.707			0.589
Tibia/femur	1.000			1.000		
Elsewhere	1.171	0.513-2.673		0.747	0.259-2.154	
Tumor stage			< 0.001			0.118
T1	1.000			1.000		
T2+T3	4.434	1.981-9.922		2.668	0.779-9.139	
Histologic grade			0.058			0.029
G1	1.000			1.000		
G2+G3	2.136	0.973-4.689		2.568	1.103-5.976	
miR-17-5p expression			< 0.001			0.002
Low	1.000			1.000		
High	15.727	4.613-53.615		10.471	2.290-47.870	

sion analysis also indicated that advanced tumor stage was a risk factor for progression-free survival (P < 0.001 and P=0.009, respectively; **Table 3**). Additionally, Kaplan-Meier, and univariate and multivariate Cox regression analysis all demonstrated that patients with low miR-17-5p expression experienced longer overall survival (P < 0.001; **Figure 2B**; P < 0.001 and P=0.002, respectively; **Table 4**). Moreover, univariate Cox regression analysis indicated that patients with high stage tumors exhibited shorter overall survival (P < 0.001), while advanced histologic grade was identified as a risk factor for overall survival by multivariate Cox regression analysis (P=0.029; **Table 4**).

Discussion

The present study found that miR-17-5p was up-regulated in the plasma of OS patients, and its overexpression was positively associated with advanced tumor stage. Moreover, miR-17-5p could identify OS patients from healthy volunteers with high specificity (97.2%) and 50% sensitivity. Further, survival analysis indicated that elevated miR-17-5p was a risk factor for both progression-free, and overall, survival.

Previous studies have reported dysregulation of various microRNAs in OS. Multiple microRNAs are down-regulated and act as tumor suppressors, including miR-152, miR-133a, miR-539, and miR-125b; another group of microRNAs, including miR-300, miR-191, and miR-421, are up-regulated and have oncogenic roles [4, 14, 16, 17, 21]. The function of miR-17-5p in malignant tumors may depend on the cellular context. Up-regulation of miR-17-5p has been described in gastric cancer, hepatocellular carcinoma, colorectal cancer, and pancreatic cancer, where it appears to act as an oncogene. In contrast, decreased expression of miR-17-5p has been reported in non-small-cell lung cancer; therefore, the molecule may have a role as a tumor suppressor in this context [22-26]. Wang et al. found that miR-17-5p expression levels were significantly elevated in OS tumors relative to those in adjacent tissues, and miR-17-5p overexpression was more frequent in OS specimens from patients with advanced clinical stage, positive for distant metastasis, and with poor responses to neoadjuvant chemotherapy [8]. Similarly, we also found that plasma miR-17-5p expression was increased in patients with advanced tumor stage. Further, ROC analysis indicated that miR-17-5p could identify OS with high specificity; however, the sensitivity of this test was relatively low, which is difficult to explain, hence further work is required to

explore this issue, since such a low sensitivity value could limit the value of miR-17-5p as a biomarker for OS screening.

Additionally, the value of miR-17-5p in prognosis assessment has previously been explored in studies of various other tumors. The levels of miR-17-5p in both tissue and serum have been identified as independent risk factors for overall survival in hepatocellular carcinoma [27, 28], and Yu et al. showed that high miR-17-5p expression was associated with poor prognosis in pancreatic cancer [29]. Moreover, colorectal cancer patients whose tumors had high miR-17-5p expression experienced shorter overall survival rates [26]. This study provides the first report that OS patients with elevated plasma miR-17-5p expression have shorter progression-free and overall survival, indicating that screening of miR-17-5p levels could allow identification of OS patients with a high risk of progression and recurrence, facilitating optimization of treatment strategies.

How miR-17-5p affects the development and progression of OS remains a matter of debate. miR-17-5p can prompt proliferation, migration, invasion, and tumorigenesis of OS cells and *BRCC2* is a direct target of miR-17-5p [8]. Although studies of miR-17-5p in OS are limited, findings from other malignant tumors may provide important clues. In gastric cancer, miR-17-5p/20a promoted tumor growth by down-regulating p21 and TP53INP1 expression in a mouse xenograft model, a negative association has also been observed between miR-17-5p/20a and TP53INP1 in gastric cancer tissues [30], and miR-17-5p may also increase gastric cancer cell proliferation via suppression of SOCS6 [23]. In breast cancer cells, miR-17-5p increased invasion and migration through suppression of HBP1 and subsequent activation of Wnt/β-catenin [31]. Moreover, in the human ovarian carcinoma cell lines, OVCAR3 and ES-2, Li et al. demonstrated that miR-17-5p acts as a pro-proliferative factor, through promotion of the G1/S transition of the cell cycle and suppression of apoptosis [19]; furthermore, miR-17-5p directly binds to the 3' UTR of *YES1* mRNA and increases its expression, and knockdown of *YES1* results in the inhibition of proliferation and induces cell cycle arrest, in these cell lines [19].

Although the results of the present study are encouraging, several limitations should be considered. First, the sample size of the study was small, and larger scale investigations are required to verify the results. Second, only OS patients without metastasis were enrolled, as the purpose of the study was to explore the efficacy of miR-17-5p in diagnostic screening. The present study showed that miR-17-5p had low sensitivity as a diagnostic biomarker; however, it performed well in assessment of prognosis. Whether miR-17-5p will function adequately in prognostic assessment of OS with metastasis remains to be determined. Third, although patients were treated according to the NCCN practice guidelines for OS, there were differences in the detailed treatment strategies used for individual patients. This factor was not taken into account in our survival analyses, and may reduce the credibility of the study to a limited extent. Fourth, all participants in this study were from a single center, hence whether they are representative of the whole population remains to be determined.

In conclusion, miR-17-5p is up-regulated in OS and acts as a risk factor for progression-free and overall survival. Overexpression of circulating miR-17-5p has the potential for use as a novel diagnostic and prognostic biomarker in OS.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Qiande Liao, Department of Orthopedics, Xiangya Hospital, No. 87 Xiangya Road, Changsha 410008, Hunan, China. Tel: 0086-18684689320; Fax: 0086-0731-8432-7224; E-mail: qiandeliaonani@163.com

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Broadhead ML, Clark JC, Myers DE, Dass CR and Choong PF. The molecular pathogenesis of osteosarcoma: a review. Sarcoma 2011; 2011: 959248.

[3] Bielack SS, Kempf-Bielack B, Delling G, Exner GU, Flege S, Helmke K, Kotz R, Salzer-Kuntschik M, Werner M, Winkelmann W, Zoubek A, Jurgens H and Winkler K. Prognostic factors in high-grade osteosarcoma of the ex-

tremities or trunk: an analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. J Clin Oncol 2002; 20: 776-790.
[4] Wang NG, Wang DC, Tan BY, Wang F and Yuan ZN. Down-regulation of microRNA152 is associated with the diagnosis and prognosis of patients with osteosarcoma. Int J Clin Exp Pathol 2015; 8: 9314-9319.
[5] Mirabello L, Troisi RJ and Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. Cancer 2009; 115: 1531-1543.
[6] Li F, Cao L, Hang D, Wang F and Wang Q. Long non-coding RNA HOTTIP is up-regulated and associated with poor prognosis in patients with osteosarcoma. Int J Clin Exp Pathol 2015; 8: 11414-11420.
[7] Ma B, Li M, Zhang L, Huang M, Lei JB, Fu GH, Liu CX, Lai QW, Chen QQ and Wang YL. Upregulation of long non-coding RNA TUG1 correlates with poor prognosis and disease status in osteosarcoma. Tumour Biol 2016; 37: 4445-55.
[8] Wang W, Zhang L, Zheng K and Zhang X. MiR-17-5p promotes the growth of osteosarcoma in a BRCC2-dependent mechanism. Oncol Rep 2016; 35: 1473-82.
[9] Li Z, Yu X, Shen J, Chan MT and Wu WK. MicroRNA in intervertebral disc degeneration. Cell Prolif 2015; 48: 278-283.
[10] Li Z, Yu X, Shen J, Wu WK and Chan MT. MicroRNA expression and its clinical implications in Ewing's sarcoma. Cell Prolif 2015; 48: 1-6.
[11] Yu X, Li Z, Shen J, Wu WK, Liang J, Weng X and Qiu G. MicroRNA-10b promotes nucleus pulposus cell proliferation through RhoC-Akt pathway by targeting HOXD10 in intervetebral disc degeneration. PLoS One 2013; 8: e83080.
[12] Li M, Yu M, Liu C, Zhu H, He X, Peng S and Hua J. MiR-34c works downstream of p53 leading to dairy goat male germline stem-cell (mGSCs) apoptosis. Cell Prolif 2013; 46: 223-231.
[13] Fu LL, Zhao X, Xu HL, Wen X, Wang SY, Liu B, Bao JK and Wei YQ. Identification of microRNA-regulated autophagic pathways in plant lectin-induced cancer cell death. Cell Prolif 2012; 45: 477-485.
[14] Zhou S, Wang B, Hu J, Zhou Y, Jiang M, Wu M, Qin L and Yang X. MiR-421 is a diagnostic and prognostic marker in patients with osteosarcoma. Tumour Biol 2016; 37: 9001-7.
[15] Bassampour SA, Abdi R, Bahador R, Shakeri M, Torkaman A, Yahaghi E and Taheriazam A. Downregulation of miR-133b/miR-503 acts as efficient prognostic and diagnostic factors in patients with osteosarcoma and these predictor biomarkers are correlated with overall survival. Tumour Biol 2015; [Epub ahead of print].
[16] Wang T, Ji F, Dai Z, Xie Y and Yuan D. Increased expression of microRNA-191 as a potential serum biomarker for diagnosis and prognosis in human osteosarcoma. Cancer Biomark 2015; 15: 543-550.
[17] Karbasy SH, Taheriazam A, Mirghasemi A, Sedaghati F, Shakeri M, Yahaghi E and Bahador R. Upregulation of miR-300 and downregulation of miR-125b act as potential predictor biomarkers in progression, metastasis, and poor prognosis of osteosarcoma. Tumour Biol 2015; [Epub ahead of print].
[18] Peng H, Ishida M, Li L, Saito A, Kamiya A, Hamilton JP, Fu R, Olaru AV, An F, Popescu I, Iacob R, Dima S, Alexandrescu ST, Grigorie R, Nastase A, Berindan-Neagoe I, Tomuleasa C, Graur F, Zaharia F, Torbenson MS, Mezey E, Lu M and Selaru FM. Pseudogene INTS6P1 regulates its cognate gene INTS6 through competitive binding of miR-17-5p in hepatocellular carcinoma. Oncotarget 2015; 6: 5666-5677.
[19] Li L, He L, Zhao JL, Xiao J, Liu M, Li X and Tang H. MiR-17-5p up-regulates YES1 to modulate the cell cycle progression and apoptosis in ovarian cancer cell lines. J Cell Biochem 2015; 116: 1050-1059.
[20] Jin YY, Andrade J and Wickstrom E. Non-specific blocking of miR-17-5p guide strand in triple negative breast cancer cells by amplifying passenger strand activity. PLoS One 2015; 10: e0142574.
[21] Mirghasemi A, Taheriazam A, Karbasy SH, Torkaman A, Shakeri M, Yahaghi E and Mokarizadeh A. Down-regulation of miR-133a and miR-539 are associated with unfavorable prognosis in patients suffering from osteosarcoma. Cancer Cell Int 2015; 15: 86.
[22] Oksuz Z, Serin MS, Kaplan E, Dogen A, Tezcan S, Aslan G, Emekdas G, Sezgin O, Altintas E and Tiftik EN. Serum microRNAs; miR-30c-5p, miR-223-3p, miR-302c-3p and miR-17-5p could be used as novel non-invasive biomarkers for HCV-positive cirrhosis and hepatocellular carcinoma. Mol Biol Rep 2015; 42: 713-720.
[23] Wu Q, Luo G, Yang Z, Zhu F, An Y, Shi Y and Fan D. MiR-17-5p promotes proliferation by targeting SOCS6 in gastric cancer cells. FEBS Lett 2014; 588: 2055-2062.
[24] Chatterjee A, Chattopadhyay D and Chakrabarti G. MiR-17-5p downregulation contributes to paclitaxel resistance of lung cancer cells through altering beclin1 expression. PLoS One 2014; 9: e95716.
[25] Yan HJ, Liu WS, Sun WH, Wu J, Ji M, Wang Q, Zheng X, Jiang JT and Wu CP. MiR-17-5p inhibitor enhances chemosensitivity to gemcitabine

via upregulating Bim expression in pancreatic cancer cells. Dig Dis Sci 2012; 57: 3160-3167.
[26] Ma Y, Zhang P, Wang F, Zhang H, Yang Y, Shi C, Xia Y, Peng J, Liu W, Yang Z and Qin H. Elevated oncofoetal miR-17-5p expression regulates colorectal cancer progression by repressing its target gene P130. Nat Commun 2012; 3: 1291.
[27] Chen L, Jiang M, Yuan W and Tang H. MiR-17-5p as a novel prognostic marker for hepatocellular carcinoma. J Invest Surg 2012; 25: 156-161.
[28] Zheng J, Dong P, Gao S, Wang N and Yu F. High expression of serum miR-17-5p associated with poor prognosis in patients with hepatocellular carcinoma. Hepatogastroenterology 2013; 60: 549-552.
[29] Yu J, Ohuchida K, Mizumoto K, Fujita H, Nakata K and 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: 748-757.
[30] Wang M, Gu H, Qian H, Zhu W, Zhao C, Zhang X, Tao Y, Zhang L and Xu W. MiR-17-5p/20a are important markers for gastric cancer and murine double minute 2 participates in their functional regulation. Eur J Cancer 2013; 49: 2010-2021.
[31] Li H, Bian C, Liao L, Li J and Zhao RC. MiR-17-5p promotes human breast cancer cell migration and invasion through suppression of HBP1. Breast Cancer Res Treat 2011; 126: 565-575.