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Original Article

Decreased serum miR-375 and miR-320a levels are useful in predicting liver cancer

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Abstract: Purpose: The aim of the current study was to explore expression levels of miR-375 and miR-320a in the plasma of patients with liver cancer, analyzing diagnostic efficacy and the effects of miR-375 and miR-320a. Methods: Blood samples from 72 patients with liver cancer and blood samples from 46 concurrent health examinations were used for prospective analysis. Patients with liver cancer were defined as the liver cancer group, while healthy physical examination patients were defined as the control group. Real-time quantitative PCR (RT-PCR) was used to measure expression of miR-375 and miR-320a in plasma. Differences in expression levels of miR-375 and miR-320a between the liver cancer group and control group were determined. Correlation levels between miR-375 and miR-320a and liver cancer incidence rates were analyzed, as well as the value of miR-375 and miR-320a for diagnosis of liver cancer. Results: Expression levels of miR-375 and miR-320a in the liver cancer group were significantly lower than those in the control group ($P<0.001$). Expression levels of miR-375 and miR-320a showed no differences in different patients in terms of age, weight, sex, place of residence, smoking habits, and exercise habits ($P>0.050$). However, they showed significant differences in different pathological stages, lymphatic metastasis, and differentiation degrees ($P<0.001$). Receiver operating characteristic curve analysis indicated that, for miR-375 expression at a cut-off value of 15.28, the sensitivity for diagnosing liver cancer was 95.22%. The specificity was 61.94%. Using 16.68 as a cut-off value for miR-320a expression, the sensitivity for diagnosing liver cancer was 98.61% and the specificity was 58.94%. Using 0.507 as a cut-off value for miR-375 and miR-320a expression, the sensitivity for combined diagnosing liver cancer was 71.53% and the specificity was 89.75%. Conclusion: The combination of miR-375 and miR-320a may become a useful indicator for diagnosis of liver cancer in the future.

Keywords: miR-375, miR-375, liver cancer, early diagnosis

Introduction

Liver cancer is one of the most common malignant tumors, with very high incidence rates [1]. According to statistics, in 2016, the number of new liver cancer patients, worldwide, reached about 2.4 million. The cumulative number of patients with liver cancer has exceeded 500 million [2, 3]. Patient populations with liver cancer have shown large regional differences. Incidence rates are more significant in some coastal countries and in Africa [4]. Currently, incidence of liver cancer is rising yearly. Some studies have estimated that by 2030, liver cancer will become the most common malignant tumor in the world [5]. Although current incidence rates of liver cancer are not the highest, the threat of liver cancer to the human body is the largest among malignant tumors. Data has shown that 5-year survival rates for liver cancer are only 10 to 30% [6]. In the face of the harm caused by liver cancer, clinicians have advocated "early detection and early treatment". However, because there are no specific symptoms in the early stages of liver cancer, patients often ignore or mishandle their treatment because of a lack of medical knowledge. Most patients have reached the middle or late stages of liver cancer at diagnosis [7]. At present, a diagnosis of liver cancer still needs to be confirmed by a pathological biopsy. Thus, there is an urgent need for a sensitive and accurate tumor examination methods, improving diagnosis rates of early liver cancer [8].

Table 1. Primer sequences

	R	F
miR-375	5'-CGCGGTTTGTTCGTTCGGCTC-3'	5'-ATCCAGTGCAGGGTCCGAGG-3'
miR-320a	5'-TATTCGCACTGGATACGACTCCAGC-3'	5'-GTCGTATCCAGTGCAGGGTCCGAGG-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'

Research has increasingly focused on the involvement of microRNAs (miRNAs) in pathological conditions. MicroRNAs are a type of conserved non-coding RNA that can regulate gene expression post-transcriptionally. They are closely related to cell growth, proliferation, and differentiation. MicroRNAs are closely related to occurrence and development of various tumors in humans [9-11]. MicroRNA-375 (miR-375) and microRNA-320a (miR-320a) have shown abnormal expression in a variety of tumors, participating in the process of tumor development [12-14]. However, their roles in liver cancer remain unclear. One study showed that miR-375 and miR-320a were included in the detection of abnormal genes in the serum of patients with liver cancer [15], but the roles of miR-375 and miR-320a in liver cancer remain unclear. Therefore, the current study aimed to evaluate the roles of miR-375 and miR-320a in occurrence and development of liver cancer, analyzing the diagnostic value for liver cancer and providing reference and guidance for diagnosis of early liver cancer in the future.

Materials and methods

Participant information

A prospective analysis was performed on 74 patients with liver cancer and 46 blood samples from concurrent health examinations of healthy subjects. Patients with liver cancer comprised the liver cancer group. The group included 42 males and 32 females, aged between 37 and 66 years old, with an average age of 52.24±10.88 years. Staging of liver cancer in patients refers to liver cancer TNN Staging [16]. Healthy physical examination subjects were used as the control group. This group included 28 males and 18 females, aged 35-68 years, with a mean age of 51.27±11.36 years. The current study was approved by the Ethics Committee and all subjects provided written informed consent.

Inclusion and exclusion criteria

Inclusion criteria: Clinical symptoms were in accordance with 2015 Liver Cancer Diagnosis guidelines [15]; Liver cancer was confirmed after biopsies in the Pathology Department; Patients were evaluated according to the TNM Staging for liver cancer [16]; After diagnosis, patients were treated with surgical resections; Patients between 30 and 70 years old; Patients that did not receive any radiotherapy, chemotherapy, or hormone therapy before surgery; Patients providing complete pathological information; Patients agreed to cooperate with the work arrangements of the medical staff in the hospital. Exclusion criteria: Patients with other tumors; Patients with cardiovascular and cerebrovascular diseases; Patients with severe organ failure; Patients with mental illness; Patients with surgical tolerance; Patients with physical disabilities; Transferred patients.

Methods

After diagnosis with liver cancer, 4 mL of venous blood was collected from the patients. After standing at room temperature for 30 minutes, the blood was centrifuged for 10 minutes at 4,000 rpm. The supernatant removed and placed in a -80°C refrigerator until testing. Levels of alpha-fetoprotein (AFP) were analyzed using an automatic biochemistry analyzer. Real-time quantitative PCR (RT-PCR) was used to measure expression of miR-375 and miR-320a in plasma. The miR-375 kit was purchased from Nanjing Kebai Biotechnology Co., Ltd. (SF-1622) and the miR-320a kit was purchased from Xiamen Keyan Co., Ltd. (XW-CPK3172). The experiment was carried out according to the instructions of the TRIzol RNA isolation and extraction kit (purchased from Chengdu Dongsheng Kechuang Technology Co., Ltd., 15596026). Plasma miRNA was isolated and purified and the RNA was reverse transcribed into cDNA, according to the instructions of the reverse transcription kit (purchased from Shanghai Haifang Biotechnology Co., Ltd., A0005). Concentration and purity levels were estimated according to an OD260/OD280 value of 1.8-2.0. The cDNA sample was placed in the refrigerator before use as a template for the RT-PCR reaction. RT-PCR primers were synthesized by Thermo Fisher Scientific, China

Table 2. Comparison of clinical data [n (%)]

	Liver cancer group (n=72)	Control group (n=46)	X^2 or t	P
Age	52.24±10.88	51.27±11.36	0.464	0.643
Body weight (KG)	63.27±15.74	64.33±16.04	0.354	0.724
Height (cm)	162.37±11.74	164.52±12.31	0.952	0.343
Gender			0.075	0.784
male	42 (58.33)	28 (60.87)		
female	30 (41.67)	18 (39.13)		
Place of residence			0.273	0.601
town	50 (69.44)	34 (73.91)		
rural	22 (30.56)	12 (26.09)		
Marital status			0.339	0.560
married	67 (93.06)	44 (95.65)		
unmarried	5 (6.94)	2 (4.35)		
Smoking			0.359	0.549
yes	43 (59.72)	30 (65.22)		
no	29 (40.28)	16 (34.78)		
Sports habit			0.093	0.761
yes	11 (15.28)	8 (17.39)		
no	61 (84.72)	38 (82.61)		
Pathological staging				
I~II	26 (36.11)	-		
III~IV	46 (63.89)	-		
Lymphatic transfer				
yes	49 (68.06)	-		
no	23 (31.94)	-		
Differentiation				
Highly differentiated	19 (26.39)	-		
Medium differentiation	30 (41.67)	-		
Low differentiation	23 (31.94)	-		

(**Table 1**). The reaction included cDNA 1 μL, forward primer 0.4 μL, universal miRNA qPCR primer 0.4 μL, 2 × TransStart Tip Green qPCR SuperMix 10 μL, passive reference dye (50 ×) (Optional) 0.4 μL, and ddH_20, to a total volume of 20 μL. Conditions for the miR-375 reaction were 95°C for 30 seconds, followed by 45 cycles of 95°C for 5 seconds and 64°C for 34 seconds. Reaction conditions for miR-320a were 95°C 30 seconds, followed by 40 cycles of 95°C for 30 seconds and 60°C for 30 seconds. Relative expression levels of miR-320a and miR-375 were expressed using the 2-ΔΔCt method, taking U6 as internal reference. All tests were repeated three times and the results were averaged.

Outcome measures

Differences in miR-375 and miR-320a expression levels between the liver cancer group and control group were determined. Values of miR-375 and miR-320a in the diagnosis of liver cancer were analyzed. They were compared with the diagnosis results of AFP (AFP >25 ug/L is regarded as having liver cancer).

Statistical methods

Data was analyzed and processed using SPSS 24.0 statistical software (Shanghai Yuchuang Network Technology Co., Ltd.). Count data, such as patient clinical data, are expressed in the form of numbers or percentages. Chi-square testing was used for comparisons between groups. Measured data, such as expression of miR-375, are expressed as the mean ± standard deviation. Analysis of variance was used to compare values between groups and post-hoc Bonferroni's t-tests were used for comparisons among groups. The diagnostic value of miR-375 and miR-320a expression was analyzed using receiver operating characteristic (ROC) curves. *P* values <0.050 indicate statistical significance.

Results

Comparison of baseline clinical data

Age, weight, height, gender, place of residence, marital status, smoking status, and exercise habits of the two groups were compared (**Table 2**). There were no significant differences (P>0.050) between the two groups concerning these variables.

Expression of miR-375 and miR-320a

The relative expression level of miR-375 in the liver cancer group was 0.69±0.08, significantly

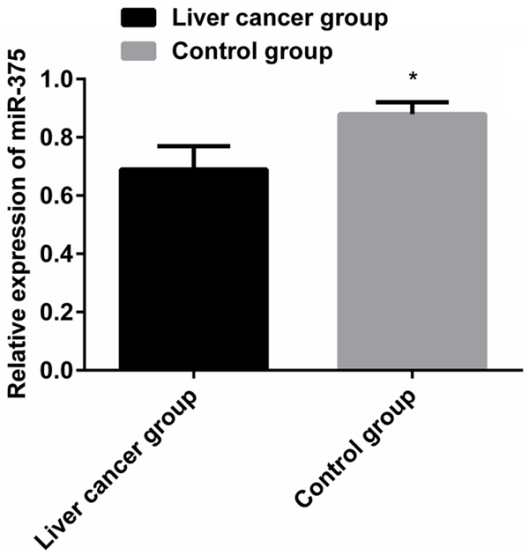

Figure 1. miR-375 expression levels in liver cancer and control groups. *P<0.001 for comparison with expression levels of miR-375 in the liver cancer group.

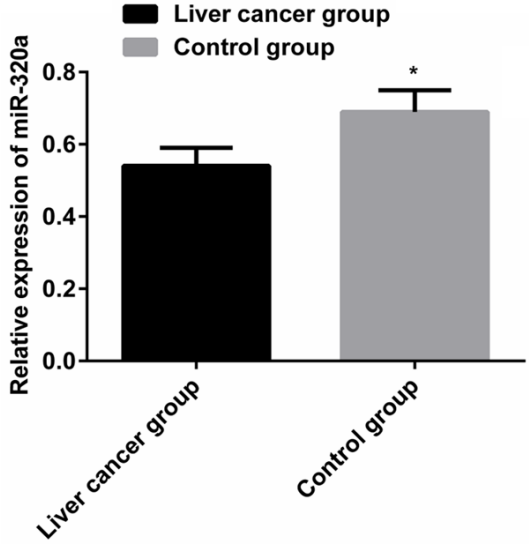

Figure 2. miR-320a expression levels in liver cancer and control groups. **P<0.001 for comparison with expression levels of miR-320a in the liver cancer group.

lower than that in the control group (0.88±0.04), P<0.001. The relative expression level of miR-320a in the liver cancer group was 0.54±0.05, also significantly lower than that in the control group (0.69±0.06), P<0.001 (**Figures 1** and **2**).

Expression patterns of miR-375 and miR-320a across clinical characteristics

Expression levels of miR-375 and miR-320a showed no differences in different patients in terms of age, weight, gender, place of residence, smoking habits, or exercise habits (P>0.050). However, they showed significant differences in different pathological stage, lymphatic metastasis, and differentiation degrees (P<0.001) (**Tables 3** and **4**).

Detection of liver cancer diagnosis efficiency using miR-375 and miR-320a

ROC curve analysis indicated that, when the cut-off value was 0.507, the sensitivity of miR-375 combined with miR-320a for diagnosis of liver cancer was 71.53% and the specificity was 89.75%. When the cut-off value was 25, the sensitivity of AFP in the diagnosis of liver cancer was 94.51% and the specificity was 73.14%. The sensitivity of AFP in the diagnosis of liver cancer was significantly higher than that of miR-375 and miR-320a. The specificity was significantly lower than that of miR-375 and miR-320a (P<0.001). (**Figure 3**, **Table 5**).

Discussion

Liver cancer is a malignant tumor with very high mortality rates. Better healing effects can usually be achieved in the early stages of tumorigenesis using resections and radiotherapy. However, because there have been no significant breakthroughs in the early diagnosis of liver cancer and early liver cancer shows no specific symptoms, most patients have developed a middle- or late-stage disease by the time liver cancer is diagnosed. At this point, the tumor is generally metastasized and invaded. This makes treatment difficult, resulting in poor prognosis [16-18]. At present, the pathogenesis of liver cancer is unclear. Early diagnosis of liver cancer lacks an effective and accurate reference index. However, the discovery of miRNAs and their association with diseases may provide a new direction for targeted research of tumors [19].

MicroRNAs are endogenously expressed non-coding small RNAs that occupy only 1% to 3% of the human genome sequence, about 17-25 nucleotides in length [20]. Incomplete pairing of the non-coding region at the 3'UTR end of the target mRNA causes blockage of transla-

Table 3. Pathological correlation between miR-375 and liver cancer

	n	miR-375	F	P
Age			0.595	0.554
>52	43	0.68±0.07		
≤52	29	0.69±0.07		
Body weight (KG)			0.496	0.623
>63	34	0.67±0.08		
≤63	38	0.68±0.09		
Gender			0.578	0.565
Male	42	0.69±0.08		
Female	30	0.68±0.06		
Place of residence			0.463	0.645
town	50	0.67±0.09		
rural	22	0.68±0.07		
Smoking			1.846	0.069
yes	43	0.62±0.13		
no	29	0.67±0.08		
Sports habit			0.496	0.621
yes	11	0.67±0.07		
no	61	0.68±0.06		
Pathological staging			6.602	<0.001
I~II	26	0.70±0.09		
III~IV	46	0.51±0.13		
Lymphatic transfer			9.350	<0.001
yes	49	0.52±0.08		
no	23	0.69±0.05		
Differentiation			218.834	<0.001
Highly differentiated	19	0.69±0.07		
Medium differentiation	30	0.54±0.02		
Low differentiation	23	0.42±0.03		

tion. Effects of miRNAs on life activities, such as apoptosis, proliferation, metastasis, and differentiation of cells, have been demonstrated to play important roles in various tumors as cancer-promoting or tumor suppressor genes [21, 22]. In the future, studies of miRNAs may become the keys to diagnosis and treatment of cancer. The current study was especially interested in miR-375 and miR-320a. miR-375 is found in the gene region of *CRYAB2* and *CCDC108* in the 2q35 region of human chromosome 2. It can generate mRNA precursors about 70 nucleotides in length under the catalysis of RNA polymerase II. It can regulate cell activation with Dicer and transactivation of responsive RNA binding proteins [23]. However, miR-320a is located on human chromosome 8 and plays a regulatory role in the proliferation of tumor cells through targeting the β-catenin mRNA [24]. miR-375 and miR-320a have been shown to play different roles in multiple tumors. However, their roles in liver cancer have not been clarified. Therefore, in the current study, expression of miR-375 and miR-320a in the blood of patients with liver cancer and in healthy controls was detected using qPCR. This study also explored the roles and diagnostic value of miR-375 and miR-320a in liver cancer, examining their relationship with pathological conditions of patients with liver cancer.

Results of this study showed that miR-375 and miR-320a expression levels were significantly lower in the patients with liver cancer, compared with healthy controls, suggesting that miR-375 and miR-320a are closely related to occurrence and development of liver cancer. They may be involved in its pathogenesis as proto-oncogenes. Present results were consistent with those of Cui et al. [25] and Wang et al. [26]. Cui et al. further mentioned that high expression of miR-375 in liver tissues could inhibit cell proliferation and migration and induce cell cycle G1 arrest and apoptosis. Zhang et al. [27] proposed that expression levels of star cell upregulated gene 1 (AGE-1) in normal human tissues is minimal. However, high expression of AEG-1 can turn non-carcinogenic hepatocytes into highly invasive hepatocytes. It was, therefore, speculated that miR-375 could reduce the carcinogenic effects of hepatocytes by inhibiting expression of AEG-1 and exerting tumor suppressing effects. Schwartzman et al. [28] showed that another driving factor in liver cancer is Yes-related protein (YAP), which is a downstream effector of the Hippo signaling pathway. It influences organ size by regulating cell proliferation and apoptosis. miR-375 binds to a specific site of the 3' non-coding region of the *YAP* proto-oncogene and regulates *YAP* expression at the post-transcriptional level. Moreover, miR-375-mediated inhibition of *YAP* translation inhibits the ability of YAP to activate hepatoma cells and controls the development of cancer. In addition, Chang [29] suggested that miR-375 might be related to autophagy. However, this has not been verified and should

Table 4. Pathological correlation between miR-320a and liver cancer

	n	miR-320a	F	P
Age			0.696	0.489
>52	43	0.53±0.07		
≤52	29	0.54±0.04		
Body weight (KG)			0.930	0.356
>63	34	0.56±0.04		
≤63	38	0.55±0.05		
Gender			0.704	0.484
male	42	0.56±0.07		
female	30	0.55±0.04		
Place of residence			1.667	0.100
town	50	0.53±0.04		
rural	22	0.55±0.06		
Smoking			1.212	0.230
yes	43	0.53±0.03		
no	29	0.52±0.04		
Sports habit			0.671	0.505
yes	11	0.56±0.07		
no	61	0.55±0.04		
Pathological staging			11.253	<0.001
I~II	26	0.58±0.07		
III~IV	46	0.42±0.05		
Lymphatic transfer			14.592	<0.001
yes	49	0.33±0.04		
no	23	0.49±0.05		
Differentiation			226.634	<0.001
Highly differentiated	19	0.53±0.04		
Medium differentiation	30	0.42±0.03		
Low differentiation	23	0.27±0.05		

be investigated in future research. Lu et al. [30] suggested that downregulation of miR-320a could activate Wnt/β-catenin signaling pathways and promote cell proliferation, suggesting that miR-320a also acts as a tumor suppressor gene in patients with cancer.

Further analysis of the clinicopathological status of patients with liver cancer with different expression levels of miR-375 and miR-320a showed that expression levels of miR-375 and miR-320a did not correlate significantly with age, weight, sex, place of residence, smoking habits, or exercise habits. However, they were closely associated with pathological stage, lymphatic metastasis, and differentiation. Results suggest that miR-375 and miR-320a are closely related to the differentiation and proliferation of hepatoma cells. The degree of disease progression can be judged by detecting expression levels of miR-375 and miR-320a.

ROC curve analysis showed that, when the cut-off value was 0.507, the sensitivity of miR-375 combined with miR-320a in the diagnosis of liver cancer was 71.53% and the specificity was 89.75%. When the cut-off value was 24.973, the sensitivity of AFP in the diagnosis of liver cancer was 94.51% and the specificity was 73.14%. The sensitivity of AFP in the diagnosis of liver cancer was significantly higher than that of miR-375 and miR-320a. Its specificity was significantly lower than that of two. The reason for this is presumed to be that AFP, as the most sensitive indicator for responding to liver function damage, provides valuable reference for the diagnosis of liver function. However, AFP detection does not have good specificity. Thus, it is impossible to accurately determine what kind of liver damage has occurred in the patient. At present, miR-375 and miR-320a are still abnormally expressed in various tumors. Thus, they cannot be completely regarded as diagnostic markers for diagnosis of liver cancer in clinical practice. The combined examination of miR-375 and miR-320a can be used as an indicator to screen early liver cancer and prevent occurrence.

Furthermore, the current study investigated expression levels of miR-375 and miR-320a in liver cancer. Results indicated that a combination of miR-375 and miR-320a might be an effective indicator for future diagnosis and treatment of liver cancer. However, this conclusion requires further in-depth experimental confirmation in a larger cohort, as the number of subjects in this study was small. In addition, expression levels of miR-375 and miR-320a in liver cancer tissues were not analyzed. The mechanisms of action of miR-375 and miR-320a in liver cancer remain unclear, requiring further research.

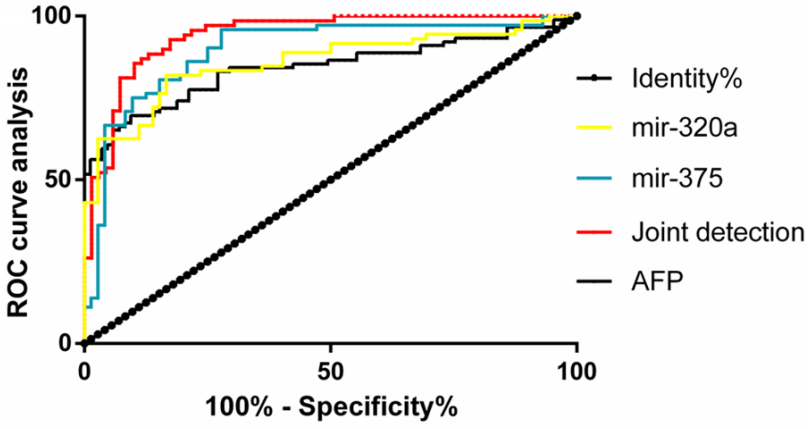

Figure 3. ROC curve analysis. When the cut-off value was 0.507, the sensitivity of miR-375 combined with miR-320a in the diagnosis of liver cancer was 71.53% and the specificity was 89.75%. When the cut-off value was 24.973, the sensitivity of AFP in the diagnosis of liver cancer was 94.51% and the specificity was 73.14%.

Table 5. Diagnostic efficacy of combined diagnosis and AFP for liver cancer

	AFP	Joint diagnosis	miR-375	miR-320a
Sensitivity (%)	94.51	71.53	95.22	98.61
Specificity (%)	73.14	89.75	61.94	58.94
cut-off	24.973	0.507	15.28	16.68
AUC	0.842	0.936	0.895	0.859
95% CI	0.781~0.903	0.90~0.96	0.840~0.951	0.796~0.922
P	<0.001	0.004	<0.001	<0.001

In summary, expression levels of miR-375 and miR-320a are significantly lower in plasma from patients with liver cancer. The combination of the two is expected to become an excellent indicator for diagnosis of liver cancer in the future.

Disclosure of conflict of interest

None.

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References

[1] Edwards BK, Ward E, Kohler BA, Eheman C, Zauber AG, Anderson RN, Jemal A, Schymura MJ, Lansdorpvogelaar I and Seeff LC. Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. Cancer 2009; 116: 544-573.

[2] Fujimoto A, Furuta M, Totoki Y, Tsunoda T, Kato M, Shiraishi Y, Tanaka H, Taniguchi H, Kawakami Y and Ueno M. Whole-genome mutational landscape and characterization of noncoding and structural mutations in liver cancer. Nature Genetics 2016; 48: 500-509.

[3] Ho MC, Hasegawa K, Chen XP, Nagano H, Lee YJ, Chau GY, Zhou J, Wang CC, Choi YR and Poon RT. Surgery for intermediate and advanced hepatocellular carcinoma: a consensus report from the 5th Asia-Pacific Primary Liver Cancer Expert Meeting (APPLE 2014). Liver Cancer 2016; 5: 245-256.

[4] Lu Z, Xiao Z, Liu F, Ming C, Li W, Zhe Y, Li J, Ye L and Zhang X. Long non-coding RNA HULC promotes tumor angiogenesis in liver cancer by up-regulating sphingosine kinase 1 (SPHK1). Oncotarget 2016; 7: 241-254.

[5] Zhu D, Tao W, Zhang H, Liu G, Wang T, Zhang L, Zeng X and Mei L. Docetaxel (DTX)-loaded polydopamine-modified TPGS-PLA nanoparticles as a targeted drug delivery system for the treatment of liver cancer. Acta Biomaterialia 2016; 30: 144-154.

[6] Park HC, Yu JI, Cheng JC, Zeng ZC, Hong JH, Wang ML, Kim MS, Chi KH, Liang P and Lee R. Consensus for radiotherapy in hepatocellular carcinoma from the 5th Asia-Pacific Primary Liver Cancer Expert Meeting (APPLE 2014): current practice and future clinical trials. Liver Cancer 2016; 5: 162-174.

[7] Sia D, Villanueva A, Friedman SL and Llovet JM. Liver cancer cell of origin, molecular class, and effects on patient prognosis. Gastroenterology 2016; 152: 745.

[8] Zheng C, Zheng L, Yoo JK, Guo H, Zhang Y, Guo X, Kang B, Hu R, Huang JY and Zhang Q. Landscape of infiltrating T cells in liver cancer revealed by single-cell sequencing. Cell 2017; 169: 1342-1356.

[9] Bracken CP, Scott HS and Goodall GJ. A network-biology perspective of microRNA function and dysfunction in cancer. Nature Reviews Genetics 2016; 17: 719.
[10] Christopher AF, Kaur RP, Kaur G, Kaur A, Gupta V and Bansal P. MicroRNA therapeutics: discovering novel targets and developing specific therapy. Perspect Clin Res 2016; 7: 68-74.
[11] Rupaimoole R and Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov 2017; 16: 203.
[12] Lu Y, Wu D, Wang J, Li Y, Chai X and Kang Q. miR-320a regulates cell proliferation and apoptosis in multiple myeloma by targeting pre-B-cell leukemia transcription factor 3. Biochem Biophys Res Commun 2016; 473: 1315-1320.
[13] Mao Q, Tao Q, Luo B, Guo X, Lei L and Zheng Q. MiR-375 targets KLF4 and impacts the proliferation of colorectal carcinoma. Tumour Biol 2016; 37: 463-471.
[14] Selth LA, Das R, Townley SL, Coutinho I, Hanson AR, Centenera MM, Stylianou N, Sweeney K, Soekmadji C and Jovanovic L. A ZEB1-miR-375-YAP1 pathway regulates epithelial plasticity in prostate cancer. Oncogene 2016; 36: 24-34.
[15] Kudo M. Surveillance, diagnosis, treatment, and outcome of liver cancer in Japan. Liver Cancer 2015; 4: 39.
[16] Gorp TV, Cadron I, Despierre E, Daemen A, Leunen K, Amant F, Timmerman D, Moor BD and Vergote I. HE4 and CA125 as a diagnostic test in ovarian cancer: prospective validation of the Risk of Ovarian Malignancy Algorithm. Br J Cancer 2011; 104: 863-870.
[17] Jin C, Shi W, Wang F, Shen X, Qi J, Cong H, Yuan J, Shi L, Zhu B and Luo X. Long non-coding RNA HULC as a novel serum biomarker for diagnosis and prognosis prediction of gastric cancer. Oncotarget 2016; 7: 51763-51772.
[18] Wentzensen N, Poole EM, Trabert B, White E, Arslan AA, Patel AV, Setiawan VW, Visvanathan K, Weiderpass E and Adami HO. Ovarian cancer risk factors by histologic subtype: an analysis from the ovarian cancer cohort consortium. J Clin Oncol 2016; 34: 2888.
[19] Erika L, Carla S, Lorea M, Ibai G, María A, María A, Caffarel MM, Araujo AM, María A and Marta FM. New concepts in cancer biomarkers: circulating miRNAs in liquid biopsies. Int J Mol Sci 2016; 17: 627.
[20] Saadatpour L, Fadaee E, Fadaei S, Mansour RN, Mohammadi M, Mousavi SM, Goodarzi M, Verdi J and Mirzaei H. Glioblastoma: exosome and microRNA as novel diagnosis biomarkers. Cancer Gene Ther 2016; 23: 415-418.
[21] Sun C, Li S, Yang C, Xi Y, Wang L, Zhang F and Li D. MicroRNA-187-3p mitigates non-small cell lung cancer (NSCLC) development through down-regulation of BCL6. Biochem Biophys Res Commun 2016; 471: 82-88.
[22] Li Z, Ma YY, Wang J, Zeng XF, Li R, Kang W, Hao XK. Exosomal microRNA-141 is upregulated in the serum of prostate cancer patients. Onco Targets Ther 2015; 9: 139-48.
[23] Yu X, Zhao W, Yang X, Wang Z and Hao M. miR-375 affects the proliferation, invasion, and apoptosis of HPV16-Positive human cervical cancer cells by targeting IGF-1R. Int J Gynecol Cancer 2016; 26: 851-858.
[24] Zhu Y, Zhang Y, Sui Z, Zhang Y, Liu M, Tang H. USP14 de-ubiquitinates vimentin and miR-320a modulates USP14 and vimentin to contribute to malignancy in gastric cancer cells. Oncotarget 2017; 8: 48725-48736.
[25] Cui F, Wang S, Lao I, Zhou C, Kong H, Bayaxi N, Li J, Chen Q, Zhu T and Zhu H. miR-375 inhibits the invasion and metastasis of colorectal cancer via targeting SP1 and regulating EMT-associated genes. Oncol Rep 2016; 36: 487-493.
[26] Wang Y, Zeng J, Pan J, Xue G, Li L, Jing W, Ping S, Ying W, Liu J and Wang L. MiR-320a inhibits gastric carcinoma by targeting activity in the FoxM1-P27KIP1 axis. Oncotarget 2016; 7: 29275-29286.
[27] Zhang X, Cai D, Meng L and Wang B. MicroRNA-124 inhibits proliferation, invasion, migration and epithelial-mesenchymal transition of cervical carcinoma cells by targeting astrocyte-elevated gene-1. Oncol Rep 2016; 36: 2321.
[28] Schwartzman M, Reginensi A, Wong JS, Basgen JM, Meliambro K, Nicholas SB, D'Agati V, Mcneill H and Campbell KN. Podocyte-Specific deletion of Yes-Associated protein causes FSGS and progressive renal failure. J Am Soc Nephrol 2015; 27: 216.
[29] Chang Y, Yan W, He X, Zhang L, Li C, Huang H, Nace G, Geller DA, Lin J and Tsung A. miR-375 inhibits autophagy and reduces viability of hepatocellular carcinoma cells under hypoxic conditions. Gastroenterology 2012; 143: 177-87, e8.
[30] Lu C, Liao Z, Cai M, Zhang G. MicroRNA-320a downregulation mediates human liver cancer cell proliferation through the Wnt/β-catenin signaling pathway. Oncol Lett 2017; 13: 573-578.