DonnishJournals

Donnish Journal of Cancer Research and Experimental Oncology
Vol 2(2) pp. 004-011 November, 2016.
http://www.donnishjournals.org/djcreo
ISSN: 2984-8970

Original Research Paper

MicroRNA-143 and -155 Levels as Biomarkers for Hepatocellular Carcinoma in Patients with Chronic Hepatitis C Virus Infection

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Accepted 10th November, 2016.

Background: **Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. It commonly develops on cirrhotic livers, and surveillance programs have therefore been suggested to identify early HCC, at a stage when it remains suitable for surgical therapy and has a better clinical outcome.** ***Aim:*** **This study aimed to investigate the association between MicroRNAs 143, 155 and prediction of HCC in Egyptian patients.** ***Methods:*** **The present study was done on a total number 141 included 47 healthy individuals as control group. Serum samples were obtained from 94 patients with chronic liver disease, divided into two groups: Group (I) included forty-seven patients with HCC, patients with cancers other than HCC or metastatic liver cancer were excluded. Group (II) included forty-seven patients with liver cirrhosis and without any evidence of HCC. Questionnaire was used to collect clinical data while Alpha-fetoprotein (AFP), serum HBsAg and anti-HCV were determined using ELISA method. The expression of miRNA was evaluated using real-time quantitative RT-PCR. Abdominal ultrasound scan was also done.** ***Results:*** **The ROC curve analysis revealed that both serum miR-143 and miR-155 could serve as valuable biomarkers for detection of HCC from healthy controls. The highest AUC to differentiate HCC patients from non-HCC was 0.804 for miR-143 and 0.885 for miR-155.** ***Conclusion:*** **This study demonstrated the beneficial role of miR-155 expression might be further evaluated as novel noninvasive diagnostic biomarkers for HCC more thanmiR-143.**

Keywords: Hepatocellular carcinoma (HCC), Liver Cirrhosis (LC), Alpha-Fetoprotein (AFP), Micro RNAs (miRNA).

INTRODUCTION

Up to 25% of patients with chronic hepatitis C virus (HCV) infection are known to develop cirrhosis after 25 to 30 years, with a 1 to 4% annual risk to develop hepatocellular carcinoma (HCC) [1]. HCC accounts for 85–90% of all primary liver cancers and ranks as the fifth most prevalent malignancy all over the world [2] and the 2nd most common cause of cancer death worldwide [3]**.** Worldwide, HCC accounts for approximately 600,000 deaths annually, with approximately half occurring in China [1]. In the United States, HCC accounts for approximately 10,000 deaths annually [4]**.**

Up to now, although serum alpha-fetoprotein (AFP) level is a useful tumor marker for the detection and monitoring of HCC, the false negative rate with AFP level alone may be as high as 40% for patients with early stage HCC. Even in the patients with advanced HCC, the AFP levels may remain normal in 15 ~ 30% of all the patients. New specific markers, such as MicroRNAs have been developed to improve the sensitivity, specificity, early detection and prediction of prognosis of HCC [1]. MicroRNAs (miRNAs) are reported as a group of small non-coding RNAs that can function as endogenous RNA interference to regulate expression of the targeted genes [5]. To date, more than 1000 human miRNAs have been identified and reported in the RNA database.

Considering that altered expression of some miRNAs contributes to human carcinogenesis, a part of these miRNAs have been reported to be useful as potential biomarkers for diagnosis, prognosis, and personalized therapy of human cancers [6]. Recently, miRNAs circulating in the blood have acted as possible early diagnostic markers for HCC.
These miRNA also could serve as indicators with respect to drug efficacy and be prognostic in HCC patients. Such biomarkers would assist stratification of HCC patients and help direct personalized therapy [7]. Involvement of miRNAs in HCC has been demonstrated, as in other cancers. HCC develops via deregulation of various molecular pathways, including p53, RAS/MAPK, PI3K/AKT/mTOR, WNT/β-catenin, MET, MYC, and transforming growth factor beta. Genetic and epigenetic

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alterations, as well as aberrant miRNA expression, can affect these crucial cancer-associated pathways [8].

Hence miRNAs expression can be modified by mutations [9], polymorphisms (SNPs) [10], transcriptional deregulation, defects in the miRNAs biogenesis machinery [11], and epigenetic changes [12], such as DNA methylation that silences genes that encode miRNAs. MiRNAs are characterized by key regulatory functions and regulate many biological processes; alterations in their expression contribute to cancer. In fact, the involvement of miRNAs in tumorigenesis and tumor progression is well established, as they can behave as tumor suppressor or promoter of oncogenesis depending on the cellular function of their targets [13]. In particular, various miRNAs were deregulated (or are aberrantly expressed) in human HCC [14].

In most cases, HCC originates on a background of cirrhosis, a chronic and diffuse hepatic disease that result from continuous liver injury and regeneration, due to different etiological factors. Different etiologies of HCC play a role in the different miRNA expression profiles. miRNA-155 was found within the BIC gene on chromosome 21 in humans. The genomic structure of human BIC consists of three exons, However, it lacks a large open reading frame and therefore its sole function may be to give rise to miR-155 encoded within exon 3. MiR-155 is involved in regulating the innate immune response. It is induced by inflammatory stimuli such as the bacterial endotoxin61 and it has been identified as a target of synthetic viral intermediates and of interferon-13, the host antiviral response cytokine.

miR-155 was significantly upregulated in chronic liver disease compared to normal liver and high miR-155 expression in non-tumoral tissues was associated with poor overall survival in HCC patient [15]. Cellular miR-155 and miRNA-18a, seem able to regulate HBV infection at the transcription level either by targeting cellular transcription factors required for HBV gene expression or by a directly binding to HBV transcripts [16]. miRNAs can target important players in DNA methylation and histone modification that play crucial roles in HBV cDNA transcription [17]. On the other hand, HBV-encodedproteins can influence cellular miRNA expression [18]. MiRNAs 21 and 155 were overexpressed in sera of patients with HCC compared to patients with chronic hepatitis C, Upregulation of miR-155 promote hepatocyte proliferation and tumor genesis by increasing Wnt signalling [19].

miR-143 is one of the most important miRNAs for HBV-HCC development. Zhao et al. [20] have measured miR-143 expression levels in p21-HBx transgenic mice at different ages during disease progression. However, minimal upregulation of miR-143 was observed in HBV-negative HCC, with or without metastasis. In our study, we measured the miRNA levels as biomarkers for hepatocellular carcinoma in patients with chronic hepatitis C virus infection.

PATIENTS AND METHODS

Patients

The present study was done on a total number 141 included 47 healthy individuals as control group. The study was approved by the Ethics and Research Committee of the National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt. Blood samples were obtained from 94 patients with chronic liver disease, divided into two groups: Group (I) included forty-sevenpatients with HCC, Patients with cancers other than HCC or metastatic liver cancer were excluded. Group (II) included forty-seven patients with liver cirrhosis and without any evidence of HCC in addition to forty-seven healthy adults were recruited as controls.

HCC was diagnosed by abdominal US and serum AFP, with or without triphasic CT scan and/or liver histopathology. AFP was assayed by an enzyme immunoassay (EIA) Kit (Roche Mannheim, Germany). The clinical/pathological data of the patients were recorded, including age, sex, viral infections {Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV)}, biochemical liver function test results, and AFP levels. Tumor characteristics were detected by Abdominal US with or without CT scan. Tumor staging was done using Tokyo staging systems}.

Blood sampling and biochemical assays

Fasting venous blood samples (5 ml) were collected by trained laboratory technicians. The blood was allowed to clot and then centrifuged at 10000 rpm for 15 min to separate the serum. Blood samples were collected in sterile tubes then separated serum and kept at -20^{o}C until used. The following biochemical tests were done for all patients groups and control group: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and direct Bilirubin, Albumin, creatinine and glucose concentrations were assayed using Beckman CX4 chemistry analyzer (NY, USA, supplied by the Eastern Co. For Eng. & Trade-Giza, Egypt). Viral infection status (HCV Ab and HBS Ag) were measured using Abbott, Ax yam (USA, Supplied by Al Kamalcompany). Serum AFP level was determined using an enzyme-linked binding protein assay kit.

Extraction of micro RNA and detection by Real-time RT-PCR analysis

All patients samples were extracted by standard protocols with red blood cell lysis, TaqMan MicroRNA Reverse Transcrpition Kit, 200 Reactions (Applied biosystems, USA) was used according to the manufacturer's instructions with modifications.

Statistical analysis

The statistical analysis in this study was performed using Statistical Program for Social Sciences (SPSS) version 15. Data were expressed as mean ± standard deviation. The t-test between two groups was used to analyze the differences between two groups. Correlation between the variables was calculated using Pearson's product moment correlation coefficient. The receiver operating characteristic curve (ROC) analysis was undertaken using the expression level for each miRNA in the serum from cases and controls to assess the diagnostic accuracy of each parameter. Using this approach, the area under the ROC (AUC) identified optimal sensitivity and specificity levels at which to distinguish normal individuals.

The following formulae are used in ROC analysis

Sensitivity = a/(a+c)
Specificity = d/(b+ d)
Diagnostic accuracy = (a+d)/(a+b+c+d)
Positive predictive value = a/(a+b)
Negative predictive value =c/(c+d)
Differential positive rate = sensitivity(%) + specificity(%) – 1.
Where: a = true positive cases, b = false positive cases.
c = false negativecases, d = true negative cases.

All the *P* values were shown two-sided and a *P* value of < 0.05 was considered statistically significant.

RESULTS

The baseline characteristics and demographic data of the all the participants were summarized in Table 1. Table 1 showed the major characteristics of the patients and controls including the numbers, the ratio of males to females as well as the age of the patients given as median and range. AST, ALT, Total bilirubin, FBS, ALB, BMI, INR, creatinine and AFP were measured in patient groups and controls. These two groups of patients shared almost the same gender and age composition. The average age was respectively 57.89 ± 11.062 and 54.55 ± 11.701 (*P* = 0.439).

Table 2 showed statistically significant differences between the ALT mean of the HCC group 58.49 ± 32.76 and the control group 31.96 ± 8.57 while as regard to AST there was statistically significant differences between HCC group102.43 ± 65.95and the control group 33.09 ± 7.23. Also, there were statistically significant differences between the Alb mean of the HCC group 2.61 ± 0.52and the control group 3.84 ± 0.2.

Also, there were statistically significant differences between the AFP mean of the HCC group 2586.22 ± 6612.77and the control group 5.8 ± 1.65. There were statistically significant differences between the ALB mean of the LC group 2.46 ± 0.76 and the control group 3.84 ± 0.2. There were statistically significant differences between the mean AFP mean of the LC group 134.62 ± 194.73 and the control group 5.8 ± 1.65. There were no statistically significant differences between the mean scores of the HCCgroupand the LC group as regard ALT, AST, T.Bil and albumin. Table 5 showed the comparison between the studied groups regarding to the liver texture, PVT spleenomegaly, hepatomegaly, focal lesions and ascitis. In our study, we found that the expression of serum miR-143was distinctly increased in HCC group compared with controls (mean ± SD:9.2±1.7 vs. 6.10±1.9, P < 0.026). The serum miR-155 was also increased in patients with HCC compared with the controls (mean ± SD: 8.77±2.1 vs5.17 0±2.4, P < 0.0117).

The ROC curve analysis was used to analyze the diagnostic accuracy of serum Micro-143 and 155

The ROC curve analysis revealed that both serum miR-143 and miR-155 could serve as valuable biomarkers for HCC from healthy controls with an AUC (the areas under the ROC curve) (Figures 1) of 0.804 (P = 0.007) and 0.855 (P = 0.0006), respectively. At the cut-off value less than 210.5 for miR-143, the sensitivity and the specificity were 85% and 61%, respectively. At the cut-off value less than 258.5 for miR-155, the sensitivity and the specificity were 86% and 78%, respectively. When the diagnostic value of miR-143 and miR-155 for HCC were considered, both of the miRNAs are potential biomarkers for the diagnosis of HCC.

DISCUSSION

Currently, the identification of HCC-specific miRNA profiles in the circulation is an emerging field of particular interest. HCC represents an extremely poor prognostic cancer that remains one of the most common and aggressive human malignancies worldwide. Over the last 2 decades, the performance of tumor markers for HCC diagnosis has not been optimal. miRNAs have been implicated in roles affecting cellular proliferation and oncogenesis. Cellular miRNAs have been linked with HCC. Their availability in the circulation makes them a potential target for early tumor detection [3]. The aim of the present study was to study the potential utility of serum miR-143 and miR-155 as noninvasive markers for diagnosis of HCV-related hepatocellular carcinoma among Egyptian patients.

In this study, we analyzed the expression of two miRNAs, miR-143 and miR-155, between the patients and healthy controls. We found that miR-143 and miR-155 expression were up-regulated in patients with HCC compared with the controls. In addition, we conducted ROC analysis to detect the potential application of miR-143 and miR-155 in the diagnoses HCC. Our results showed that miR-143 and miR-155 might be potential biomarkers for both hepatitis and HCC. Our current data indicated that serum miR-143 and miR-155 expression might be further evaluated as novel noninvasive diagnostic biomarkers for HCC.

Nowadays, there are increasing studies being conducted to identify specific circulating miRNAs in the diagnosis of HCC. Fu et al. [21] conducted a study to investigate the potential of certain serum/plasma miRNAs as novel non-invasive biomarkers for early diagnosis of HBV-related HCC. Through comprehensive tests of 94 plasma samples (28 control and 66 patient plasma samples), a series of related miRNAs were reported in the study and the expression of miR-223 was the most significant. Microvesicles (MVs) packaged with miRNAs were reported to be released mainly from tumor cells. Sun et al. [22] conduct expression profiles to detect the differently expressed miRNAs. The results showed that a total of 242 aberrantly expressed miRNAs were identified in HCC-MVs compared with CHB-MVs and the control. Among them, 115 miRNAs were up-expressed with up to 31 fold difference (miR-671-5p) and 127 were down-expressed with up to 0.041 fold difference (miR-432) in HCC [22].

In this present study, we focus on the diagnostic value of miR-143 and miR-155 for chronic hepatitis and HCC. Previous reports have shown that the expression of miR-143 is extremely down-regulated in colorectal cancer, lung, bladder, and gastric cancers [23]. While it is reported the expression of miR-143 is up-regulated in pancreatic stellate cells cancer and esophageal cancer [24]. There are few reports about the relationship between miR-143 expression and the diagnosis of HCC. Up to now, only one study reported the association between miR-143 and HCC. Zhang et al. reported that the levels of miRNA-143 (miR-143) are dramatically increased in metastatic HBV-HCC of both p21-HBx transgenic mice and HCC patients. Advanced study showed that up-regulation of miR-143 expression promotes cancer cell invasion/migration and tumor metastasis by repression of FNDC3B expression [25].

miR-155 appears to be involved in tumor progression through the inhibition of multiple tumor suppressor genes, such as sex-determining region Y-gene related high-mobility-group box gene [26] and suppressor in cytokine signaling 1 [27], thus promoting proliferation and invasion in HCC. The present study revealed that miR-155 expression levels were enhanced in HCC tissues, consistent with previous findings by Hu et al [28]. However, the results of the present study were inconsistent with the findings of Han et al [29], where an association between miR-155 expression and HCC tissue differentiation was observed, as the present study did not compare the differential expression in high and low HCC differentiation groups.

Table 1: Major characteristics of the patients groups and the control

Group	Number	M/F	Median age (yrs)	Range
HCC	47	30/17	38	19-77
LC	47	11/36	59	48-89
Control	47	20/27	56	28-80

Table 2: T-test and p-value between Control and group I (HCC)

	Group	N	Mean ±Std. Deviation	Mean Difference	T	Sig. (2-tailed)
Sex	HCC	47	1.77 ± 0.43	0.4	4.281	.000
	control	47	1.36 ± 0.49			
Age	HCC	47	60.72 ± 8.74	16.3	5.788	.000
	control	47	44.43 ± 17.21			
ALT	HCC	47	58.49 ± 32.76	26.53	5.372	.000
	control	47	31.96 ± 8.57			
AST	HCC	47	102.43 ± 65.95	69.34	7.165	.000
	control	47	33.09 ± 7.23			
T.Bil	HCC	47	6.56 ± 14.67	5.77	2.698	.008
	control	47	0.79 ± 0.18			
Glucose	HCC	47	221.3 ± 114.24	119.02	7.077	.000
	control	47	102.28 ± 15.6			
Alb	HCC	47	2.61 ± 0.52	-1.22	-15.101	.000
	control	47	3.84 ± 0.2			
AFP	HCC	47	2586.22 ± 6612.77	2580.42	2.675	.009
	control	47	5.8 ± 1.65			
BMI	HCC	47	25.99 ± 2.1	2.99	4.690	.000
	control	47	23 ± 3.84			
INR	HCC	47	1.48 ± 0.32	0.5	10.537	.000
	control	47	0.98 ± 0.06			
Creatnine	HCC	47	2.09 ± 1.75	1.15	4.496	.000
	control	47	0.94 0.16			

Table 3: T-test and p-value between Control and group I (LC)

	Group	N	Mean ±Std. Deviation	Mean Difference	t	Sig. (2-tailed)
Sex	LC	47	1.57 ± 0.5	0.21	2.093	.039
	Control	47	1.36 ± 0.49			
Age	LC	47	54.02 ± 9.68	9.6	3.331	.001
	Control	47	44.43 ± 17.21			
ALT	LC	47	61.38 ± 74.42	29.43	2.693	.008
	Control	47	31.96 ± 8.57			
AST	LC	47	97.98 ± 158.42	64.89	2.805	.006
	Control	47	33.09 ± 7.24			
T.Bil	LC	47	5.29 ± 5.82	4.5	5.300	.000
	Control	47	0.79 ± 0.18			
Glucose	LC	47	201.7 ± 77.03	99.43	8.673	.000
	Control	47	102.28 ± 15.6			
Alb	LC	47	2.46 ± 0.76	-1.38	-12.044	.000
	Control	47	3.84 ± 0.2			
AFP	LC	47	134.62 ± 194.73	128.82	4.535	.000
	Control	47	5.8 ± 1.65			
BMI	LC	47	26.95 ± 2.86	3.95	5.663	.000
	Control	47	23 ± 3.84			
INR	LC	47	1.53 ± 0.77	0.54	4.831	.000
	Control	47	0.98 ± 0.06			
creatnine	LC	47	1.7 ± 1.31	0.76	3.947	.000
	Control	47	0.94 0.16			

Table 4: T-test and p-value between group I (HCC) and group II (LC)

	Group	N	Mean ±Std. Deviation	Mean Difference	t	Sig. (2-tailed)
Sex	HCC	47	1.77 ± 0.43	0.19	1.995	.049
	LC	47	1.57 ± 0.5			
Age	HCC	47	60.72 ± 8.74	6.7	3.524	.001
	LC	47	54.02 ± 9.68			
ALT	HCC	47	58.49 ± 32.76	-2.9	-.244	.808
	LC	47	61.38 ± 74.42			
AST	HCC	47	102.43 ± 65.95	4.45	.178	.859
	LC	47	97.98 ± 158.42			
T.Bil	HCC	47	6.56 ± 14.67	1.28	.554	.581
	LC	47	5.29 ± 5.82			
Glucose	HCC	47	221.3 ± 114.24	19.6	.975	.332
	LC	47	201.7 ± 77.03			
Alb	HCC	47	2.62 ± 0.52	0.16	1.188	.238
	LC	47	2.46 ± 0.76			
AFP	HCC	47	2586.22 ± 6612.77	2451.6	2.541	.013
	LC	47	134.62 ± 194.73			
BMI	HCC	47	25.99 ± 2.1	-0.96	-1.852	.067
	LC	47	26.95 ± 2.86			
INR	HCC	47	1.48 ± 0.32	-0.04	-.368	.714
	LC	47	1.53 ± 0.77			
creatnine	HCC	47	2.1 ± 1.75	0.39	1.240	.218
	LC	47	1.7 ± 1.31			

Table 5: Radiological examination of studied groups (Control, CLD, LC and HCC patients) Sonar and Computed tomography (CT)

Parameters	Control N (%)	LC N (%)	HCC N (%)	P=value
Liver: -Normal liver -Bright liver -Coarse liver	 47(100%) 0(0%) 0(0%)	 0(0%) 0(0%) 47(100%)	 0(0%) 0(0%) 47(100%)	P<0.001*
Focal lesion	0(0%)	0(0%)	47(100%)	
Ascitis: No Mild Mod Severe	 47(100%) 0(0%) 0(0%) 0(0%)	 28(60%) 11(23.3%) 4(10%) 4(6.7%)	 37(80%) 4(6.7%) 4(10%) 2(3.3%)	P=0.008*
PVT: Yes No	 0(0%) 47(100%)	 4(10%) 43(90%)	 4(10%) 43(90%)	P=0.19
Splenomegaly: Yes No	 0(0%) 47(100%)	 4(10%) 43(90%)	 10(23.3%) 37(76.7%)	P=0.01*
Hepatomegaly: Yes No	 0(0%) 47(100%)	 7(13.3%) 40(86.7%)	 4(10%) 43(90%)	P=0.12
Hypertension: Yes No	 0(0%) 47 (100%)	 4(10%) 43(90%)	 0(0%) 47(100%)	P=0.052

*p-value < 0.05 significant, PVT (portal vein thrombosis).

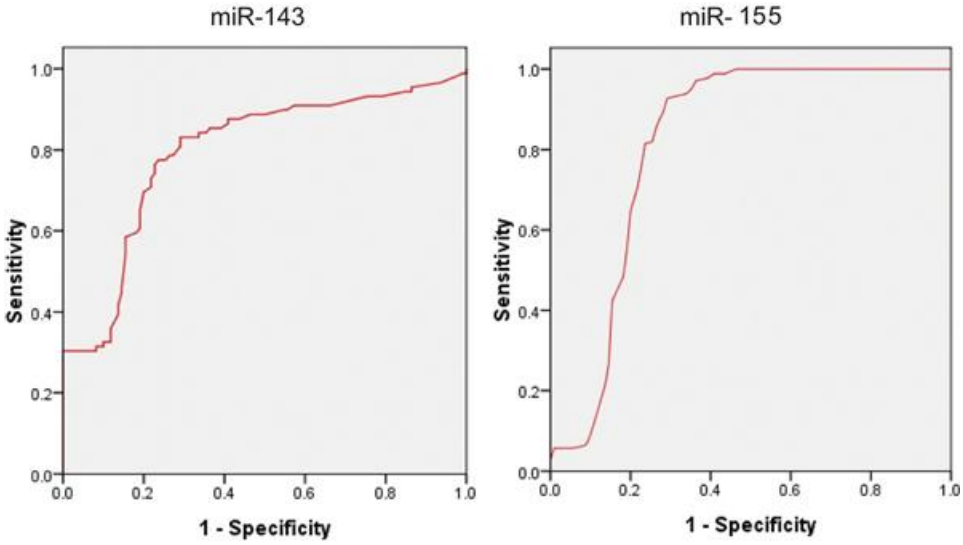

Table 6: Micro RNAs in HCC and Non-HCC Groups

	HCC Mean ± SD	Non-HCC Mean ± SD	**P value**
143 expression	9.2±1.7	6.10±1.9	0.026
155 expression	8.77±2.1	5.170±2.4	0.0117

Table 7: Correlation between the five markers according to the Roc Curve and the Area under the Curve

	Area	P- value	Cut off	Sensitivity %	Specificity %
AFP	0.778	<0.001	17	70%	61%
143	0.804	<0.001	210.5	85%	61%
155	0.855	<0.001	258.5	86%	78%

Recent years, more and more studies raised diagnostic and prognostic application of miRNAs in different diseases, including cancer. Our results showed that serum miR-143 and miR-155 were commonly up-regulated in chronic hepatitis and HCC patients, suggesting miR-143 and miR-155 may be new potential diagnostic biomarkers and targets of chronic hepatitis and HCC.

CONCLUSION

This study demonstrated the beneficial role of miR-155 expression might be further evaluated as novel noninvasive diagnostic biomarkers for HCC more than miR-143.

COMPETING INTERESTS

Authors have declared that no competing interests exist. The authors alone are responsible for the content and writing of the paper. The authors did not receive any funds from any source

REFERENCES

1. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of Hepatology. 2009;49:1335-74.hepatitis C: an update.
2. Yamazaki K, Masugi Y, Sakamoto M: Molecular pathogenesis of hepatocellular carcinoma: altering transforming growth factor-beta signaling in hepatocarcinogenesis. Dig Dis 2011, 29:284–288.
3. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA: a Cancer Journal for Clinicians. 2011;61(2):69-90
4. El-Serag HB. Hepatocellular carcinoma: Recent trends in the united states. Gastroenterology. 2004;127(5, Suppl 1):S27-S34.
5. Su ZX, Zhao J, Rong ZH, Geng WM, Wu YG, Qin CK. Upregulation of microRNA-25 associates with prognosis in hepatocellular carcinoma. Diagn Pathol. 2014;9:47.
6. Zhao J, Lu Q, Zhu J, Fu J, Chen YX. Prognostic value of miR-96 in patients with acute myeloid leukemia. Diagn Pathol. 2014;9:76.
7. Moss EG. (2003). Trends Biotechnol 21: 185–187.
8. Negrini M, Gramantieri L, Sabbioni S, Croce CM. microRNA involvement in hepatocellular carcinoma. Anticancer Agents Med Chem. 2011;11(6):500–521.
9. Yang, J.; Zhou, F.; Xu, T.; Deng, H.; Ge, Y.Y.; Zhang, C.; Li, J.; Zhuang, S.-M. Analysis of sequence variations in 59 microRNAs in hepatocellular carcinomas. Mutat. Res. 2008, 638, 205–209.
10. Akkiz, H.; Bayram, S.; Bekar, A.; Akgollu, E.; Ulger, Y. A functional polymorphism in pre-microRNA-196a-2 contributes to the susceptibility of hepatocellular carcinoma in a Turkish population: A case–control study. J. Viral Hepat. 2011, 18, e399–e407.
11. Sekine, S.; Ogawa, R.; Ito, R.; Hiraoka, N.; McManus, M.T.; Kanai, Y.; Hebrok, M. Disruption of Dicer1 induces dysregulated fetal gene expression and promotes hepatocarcinogenesis. Gastroenterology 2009, 136, 2304–2315.
12. Iorio, M.V.; Piovan, C.; Croce, C.M. Interplay between microRNAs and the epigenetic machinery: An intricate network. Biochim. Biophys. Acta 2010, 1799, 694–701.
13. Lujambio, A.; Lowe, S.W. The microcosmos of cancer. Nature 2012, 482, 347–355.
14. Otsuka, M.; Kishikawa, T.; Yoshikawa, T.; Ohno, M.; Takata, A.; Shibata, C.; Koike, K. The role of microRNAs in hepatocarcinogenesis: Current knowledge and future prospects. J. Gastroenterol. 2014, 49, 173–184.
15. Budhu, A.; Yu, Z.; Forgues, M.; Tang, ZY.; Croce, C.; Wang, XW. Immune cell-related MICRORNA-155 is associated with human liver cirrhosis and hepatocellular carcinoma. ILCA Annual Conference (abstract); Milan, Italy. Sept 4–6, 2009
16. Liu, W.H.; Yeh, S.H.; Chen, P.J. Role of microRNAs in hepatitis B virus replication and pathogenesis. Biochim. Biophys. Acta 2011, 1809, 678–685.
17. Zhang, X.; Zhang, E.; Ma, Z.; Pei, R.; Jiang, M.; Schlaak, J.F.; Roggendorf, M.; Lu, M. Modulation of hepatitis B virus replication and hepatocyte differentiation by MicroRNA-1. Hepatology 2011, 53, 1476–1485.
18. Wang, Y.; Lu, Y.; Toh, S.T.; Sung, W.-K.; Tan, P.; Chow, P.; Chung, A.Y.F.; Jooi, L.L.P.; Lee, C.G.L. Lethal-7 is down-regulated by the hepatitis B virus x protein and targets signal transducer and activator of transcription 3. J. Hepatol. 2010, 53, 57–66.
19. Zhang, Y.; Wei, W.; Cheng, N.; Wang, K.; Li, B.; Jiang, X.; Sun, S. Hepatitis C Virus-induced up-regulation of miR-155 promotes hepatocarcinogenesis by activating Wntsignaling. Hepatology 2012, 56, 1631–1640.
20. Yin Y, Zhao Y, Wang J, et al. antiCODE: a natural sense-antisense transcripts database. BMC Bioinformatics, 2007, 8: 319
21. Fu Y, Wei X, Tang C, Li J, Liu R, Shen A, Wu Z. Circulating microRNA-101 as a potential biomarker for hepatitis B virus-related hepatocellular carcinoma. Oncol Lett. 2013;6:1811–1815.
22. Sun L, Hu J, Xiong W, Chen X, Li H, Jie S. MicroRNA expression profiles of circulating microvesicles in hepatocellular carcinoma. Acta Gastroenterol Belg. 2013;76:386–392.
23. Naito Y, Sakamoto N, Oue N, Yashiro M, Sentani K, Yanagihara K, Hirakawa K, Yasui W. MicroRNA-143 regulates collagen type III expression in stromal fibroblasts of scirrhous type gastric cancer. Cancer Sci. 2014;105:228–235.
24. Liu SG, Qin XG, Zhao BS, Qi B, Yao WJ, Wang TY, Li HC, Wu XN. Differential expression of miRNAs in esophageal cancer tissue. Oncol Lett. 2013;5:1639–1642.
25. Zhang X, Liu S, Hu T, He Y, Sun S. Up-regulated microRNA-143 transcribed by nuclear factor kappa B enhances hepatocarcinoma metastasis by repressing fibronectin expression. Hepatology.2009;50:490–499.
26. Xie Q, Chen X, Lu F, et al: Aberrant expression of microRNA 155 may accelerate cell proliferation by targeting sex-determining region Y box 6 in hepatocellular carcinoma. Cancer 118: 2431-2442, 2012.
27. Yan XL, Jia YL, Chen L, et al: Hepatocellular carcinoma-associated mesenchymal stem cells promote hepatocarcinoma progression: Role of the S100A4-miR155-SOCS1-MMP9 axis. Hepatology 57: 2274-2286, 2013.
28. Hu YH, Cai Q and Lan QY: Expression of miR-155 in hepatocellular carcinoma and its effect on the proliferation of hepatocellular carcinoma cells. World J Gastroenterol 19: 1737-1741, 2012.
29. Han ZB, Chen HY, Fan JW, et al: Up-regulation of microRNA-155 promotes cancer cell invasion and predicts poor survival of hepatocellular carcinoma following liver transplantation. J Cancer Res Clin Oncol 138: 153-161, 2012.