Int J Clin Exp Pathol 2016;9(2):920-929
www.ijcep.com /ISSN:1936-2625/IJCEP0016585

Original Article

Downregulation of microRNA-542-5p and its relationship with EGFR and clinicopathological features in hepatocellular carcinoma

Jian-Jun Li[1*], Xia Yang[2*], Xiao-Miao Lin[3], Rong-Quan He[4], Xing-Gu Lin[4], Yi-Huan Luo[2], Han-Lin Wang[2], Fei-Fei Luo[2], Xin Wen[2], Yi-Wu Dang[2], Gang Chen[2]

Departments of [1]General Surgery, Western Branch, [2]Pathology, First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region, P. R. China; [3]Department of Children Rehabilitation Medicine, Guangxi Maternal and Child Health Hospital, Nanning, Guangxi Zhuang Autonomous Region, P. R. China; [4]Center for Genomic and Personalized Medicine, Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region, P. R. China. [*]Equal contributors.

Received September 21, 2015; Accepted December 5, 2015; Epub February 1, 2016; Published February 15, 2016

Abstract: Aim: To explore the expression of miR-542-5p in Hepatocellular Carcinoma (HCC), and to investigate the relationship between expressions of miR-542-5p and its potential target gene EGFR, as well as the clinical significance of miR-542-5p in HCC. Materials and methods: One hundred and one paraffin-embedded samples from primary surgical resection of HCC with complete clinical data were selected from the First Affiliated Hospital, Guangxi Medical University, China. The expression of miR-542-5p in HCC and adjacent noncancerous tissues was detected by quantitative real-time RT-PCR. Then the relation between patients' age, tumor grade, TNM stage, lymph node metastasis, recurrent event and miR-542-5p expression was analyzed. The expression of EGFR was detected with immunohistochemistry. Besides, we performed in silico prediction of the complementarity of miR-542-5p sequence to EGFR with Targetscan, miRTar and miRanda, respectively. Results: The expression of miR-542-5p in HCC tissues was 2.3028±1.26610, significantly lower than that in adjacent noncancerous tissues (3.3767±2.58785, P=0.001). The area under curve (AUC) of low miR-542-5p expression to diagnose HCC was 0.603 (95% CI: 0.524-0.681, P=0.012) with a cut-off value 1.990 evaluated by the mean expression of miR-542-5p in both HCC and adjacent noncancerous tissues. The expression of miR-542-5p was negatively correlated with tumor nodes number (r=-0.278, P=0.005), clinical TNM stage (r=-0.233, P=0.019), metastasis (r=-0.221, P=0.026), tumor capsular infiltration (r=-0.202, P=0.043), and tumor embolus in portal vein (r=-0.212, P=0.033). A negative correlation was found between the expression levels of EGFR and miR-542-5p (r=-0.139, P=0.049), which was consistent with in silico prediction. Additionally, the recurrent time of lower miR-542-5p expression group was 47.653±2.937 months, slightly shorter than that in the high expression group (63.400±3.095 months), however, no significant difference was noted (Chi-square=3.284, P=0.070). Conclusion: MiR-542-5p may play a vital role in the carcinogenesis and progression of HCC. There possibly exists a complementary targeting relationship between EGFR and miR-542-5p.

Keywords: Hepatocellular carcinoma, miR-542-5p, EGFR, quantitative real-time PCR, immunohistochemistry, recurrence

Introduction

Hepatocellular carcinoma (HCC), the most frequent primary liver malignancy, is one of the most prevalent tumors all over the world. HCC also leads as the third most common cause of cancer-related deaths [1-3]. Regardless of the progress of novel therapeutic strategies, the prognosis of advanced HCC remains poor, with a life expectancy of about half of a year from the time of diagnosis [4]. Although developed diagnostic techniques have contributed to the opportunity for more HCC patients to undergo curative surgery at early stages, the tumor recurrence and mortality rates keep still high as a result of its aggressive behaviors and limited response to adjunctive therapies in advanced stages [1, 5], the estimated recurrence rate can be as high as 70%-80% at five years [6]. Hence, there has been great interest in evaluating factors that influence prognosis of HCC.

Since the molecular biology technologies are growing in leaps and bounds, a large number of

molecular markers closely related to HCC recurrence, metastasis and survival have been discovered. New perspectives in cancer treatment have grown up recently with the springing up of the microRNAs (miRNAs). MiRNAs are a class of small non-coding RNAs of 20-24 nucleotides length which regulate gene expression at a post-transcriptional level via mRNA degradation or inhibition of protein translation. The target is located in the 3' end of non-coding position and exon area [6]. In recent years, a growing number of studies have shown that miRNAs play a similar role as proto-oncogenes or tumor suppressor genes in malignant tumor occurrence and development. Recent studies [7-9] have demonstrated that miR-542-5p was highly associated with occurrence, development process and poor clinical outcome of various forms of tumor. The study of Althoff et al. [10] found that miR-542-5p expression was inversely correlated with poor prognosis in neuroblastoma patients. MiR-542-5p was downregulated in several types of tumors [11], suggesting that it may be a hopeful tumor suppressor gene. Furthermore, Yamaguchi et al. [12] reported an inverse association of miR-542-5p level and EGFR protein in human lung cancer samples, proposing that miR-542-5p could directly target EGFR mRNA. MiRanda prediction also confirmed EGFR as one of the targets of miR-542-5p. However, the role and mechanism of miR-542-5p remains little known. Herein, we detected miR-542-5p expression in HCC by RT-qPCR and analyzed the correlation of miR-542-5p expression and clinicopathological features, including the EGFR status.

Materials and methods

Patients and specimens

We used archived formalin-fixed, paraffin-embedded (FFPE) tissues from 101 patients (80 males and 21 females) who underwent primary surgical resection of HCC at the First Affiliated Hospital, Guangxi Medical University. Survival information for 70 cases was successfully collected. HCC was diagnosed histopathologically. None of these patients had received any preoperative chemotherapy or radiotherapy. Tumor stages were classified according to the WHO tumor-nodes-metastasis (TNM) criteria. Twenty-five patients were at stage I~II and 76 patients at stage III~IV. The recurrence free survival (RFS) was defined as the length of time between the surgery and recurrence. And 101 patients adjacent normal HCC tissues were individually enrolled in the study as internal controls. The study was approved by the Research Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, China. Informed written consents were obtained from all patients who participated in this study.

Real-time RT-qPCR

Total RNAs were isolated from HCC and adjacent normal liver FFPE tissues with RNeasy FFPE Kit (QIAGEN, Germany), abiding by the manufacturer protocol. Reverse transcription (RT) and qPCR kits were used to assess expression level of miR-542-5p. Real-time RT-qPCR for miRNA was performed with Applied Biosystems PCR7900 in triplicate. The miR-542-5p abundance in each sample was normalized to its references RNU6B and RNU48. The sequences in the current study were as follows: miR-542-5p (Applied Biosystems cat. No. 4427975-001288): 5'-UCGGGGAUCAUCAUGUCACGAG-3'; RNU6B (Applied Biosystems cat. No. 4427975-001093): CGCAAGGAUGACACGCAAAUUCGUGAAGCGUUCCAUAUUUUU; RNU48 (Applied Biosystems cat. No. 4427975-001006): GAUGACCCCAGGUAACUCUGAGUGUGUCGCUGAUGCCAUCACCGCAGCGCUCUGACC. The expression of miR-542-5p was calculated with the formula 2-Δcq [13-17].

Immunohistochemistry

The immunostaining was performed according to standard SP methods from the manufacturer's protocol. Five μm thick sections were cut from each sample. The dewaxing and rehydration of the sections was followed by heat-induced epitope retrieval in citrate buffer of pH6 for half an hour (Zhongshan Jinqiao, Beijing, China). Monoclonal antibody anti-EGFR (clone EGFR, EP38Y, MAIXIN-BIO, Fuzhou, China) was incubated for 60 minutes in room temperature. 3,3 diamino-benzidine dyhidrochloride was applied as chromogen and hematoxylin was used for counterstain. The results of staining were evaluated by each author and a final agreement regarding controversial cases was reached at a multi-headed microscope. EGFR expression was classified according to intensity of staining, the staining was graded from - to 3+. No staining was recorded as -, and

Table 1. Relationship between miR-542-5p expression and clinicopathological parameters in HCC

Clinicopathological Feature		miR-542-5p relative expression (2-ΔCq)			
		n	Mean ± SD	t	P
Tissue	Adjacent non-cancerous liver	101	3.3767±2.58785	3.529[a]	0.001
	HCC	101	2.3028±1.26610		
Age	≥ 50	50	2.4245±1.28836	0.976	0.332
	< 50	51	2.1786±1.24359		
Gender	male	80	2.3965±1.29452	1.460	0.147
	female	21	1.9457±1.10756		
Differentiation	high	7	2.6657±1.58050	F=0.329[b]	0.720
	moderate	64	2.2956±1.22349		
	low	30	2.2333±1.31222		
Size	< 5 cm	18	2.0733±1.11162	-0.847	0.399
	≥ 5 cm	83	2.3525±1.29793		
Tumor nodes	single	57	2.6349±1.44586	3.347	0.001
	multi	44	1.8725±0.81836		
Metastasis	Without metastasis	49	2.5588±1.36038	2.002	0.048
	With metastasis	52	2.0615±1.13095		
Clinical TNM stage	I~II	25	2.8240±1.42018	2.430	0.017
	III~IV	76	2.1313±1.17114		
Portal vein tumor embolus	-	69	2.4851±1.35486	2.164	0.033
	+	32	1.9097±0.95442		
Vaso-invasion	-	63	2.4071±1.29146	1.067	0.288
	+	38	2.1297±1.21997		
Tumor capsular infiltration	With complete capsule	49	2.4867±1.19158	1.425	0.157
	No capsule or infiltration	52	2.1294±1.32047		
HCV	-	67	2.1939±1.28188	-1.216	0.227
	+	34	2.5174±1.22459		
HBV	-	19	2.1468±1.14595	-0.594	0.554
	+	82	2.3389±1.29621		
AFP	-	46	2.3365±1.16662	0.945	0.347
	+	39	2.1023±1.10336		
Cirrhosis	-	54	2.4843±1.50119	1.555	0.123
	+	47	2.0943±0.89732		
nm23	-	23	2.4270±1.31538	0.533	0.595
	+	78	2.2662±1.25758		
MTDH	-	38	2.2761±1.22786	-0.327	0.745
	+	51	2.3686±1.38698		
P53	-	45	2.1100±1.11224	-1.378	0.171
	+	56	2.4577±1.36750		
P21	-	67	2.4113±1.37991	1.213	0.228
	+	34	2.0888±0.98903		
VEGF	-	29	2.3600±1.31384	0.287	0.775
	+	72	2.2797±1.25504		
Ki-67 LI	Low	47	2.1640±1.17428	-0.977	0.331
	High	48	2.4223±1.39133		
MVD	Low	50	2.1682±1.12807	-1.058	0.292
	High	51	2.4347±1.38674		

[a]: Paired student's t-test was performed. [b]: One-way analysis of variance (ANOVA) test was performed.

Table 2. Relationship between miR-542-5p and EGFR expression in HCC and adjacent noncancerous tissues

		miR-542-5p relative expression (2-ΔCq)			
		n	Mean ± SD	F	P
EGFR	-	49	3.1865±2.15779	2.081	0.104
	+	69	3.0293±2.49993		
	++	45	2.7693±1.85443		
	+++	39	2.1500±1.27028		

One-way analysis of variance (ANOVA) test was performed. LSD test was performed for multiple comparisons. - vs. +: *P*=0.687. - vs. ++: *P*=0.334. - vs. +++: *P*=0.022. + vs. ++: *P*=0.516. + vs. +++: *P*=0.037. ++ vs. +++: *P*=0.176.

less than 10% tumoral cells were stained; 1+ meant a weak membrane staining, and 10%-50% weak positivity tumoral cells; 2+ was regarded as membrane staining more intense than in 1+; and 10%-50% strong positivity tumoral cells, intense staining was considered as 3+, and more than 50% positive tumoral cells were stained.

MiRNA target prediction

To determine the potential target genes, we compared the complementarity of miR-542-5p sequence to the 3'-untranslated region of EGFR with Targetscan (http://www.targetscan.org/), miRTar (http://mirtar.mbc.nct u.edu.tw/human/) and miRanda (http://www.microrna.org/microrna/ho me.do), respectively.

Statistical analysis

We employed SPSS 20.0 for statistical analysis. All data were expressed as mean ± standard error of mean. Significance of difference between two groups was analyzed by Student's t test. Correlation between miR-542-5p expression and clinicopathological parameters was detected with Spearman, including age, gender, size, tumor node, distant metastasis, clinical TNM stage, portal vein tumor embolus, vaso-invasion and tumor capsular infiltration. Significance of difference within three groups (we divided differentiation condition into three groups) was analyzed by One-way analysis of variance (ANOVA) test. Effectiveness of miR-542-5p to distinguish HCC from non-cancerous liver tissues was generated by receiver operating characteristic (ROC) curves. The log-rank test was applied to compare the recurrence between groups. And *P*-value less than 0.05 were considered statistically significant.

Results

Value of miR-542-5p in the diagnosis of HCC

The clinicopathological features of 101 HCC patients were shown in **Tables 1** and **2**. All patients only underwent liver resection and nobody received any local ablative therapy before liver resection. There was a significant difference of relative miR-542-5p expression between HCC and the paired adjacent noncancerous liver tissues. The expression of miR-542-5p in HCC tissues was 2.3028±1.26610, significantly lower than in adjacent non-cancerous tissues (3.3767±2.58785, P=0.001, **Figure 1A**). And the area under curve (AUC) of low miR-542-5p expression to diagnose HCC was 0.603 (95% CI: 0.524-0.681, P=0.012) with a cut-off value 1.990 evaluated by the mean expression of miR-542-5p in both HCC and adjacent noncancerous tissues (**Figure 1B**).

Relationship between miR-542-5p expression and clinicopathological parameters in HCC

With respect to the association between miR-542-5p expression and clinicopathological parameters of HCC, the following results were obtained (**Figure 2**). The relative expression of miR-542-5p in HCC patients with single tumor nodes (2.6349±1.44586) was prominently higher than those with multiple tumor nodes (1.8725±0.81836, P=0.001). Compared to those with metastasis (2.0615±1.13095), the level of miR-542-5p was higher in those HCC patients without metastasis (2.5588±1.36038, P=0.048). When compared with HCC patients of advanced stages (III and IV, 2.1313±1.17114), the relative expression of miR-542-5p in early stages patients (I and II, 2.8240±1.42018, P=0.017) was notably increased. Besides, the relative expression of miR-542-5p in those HCC patients with negative portal vein tumor embolus (2.4851±1.35486) was higher than those with positive portal vein tumor embolus (1.9097±0.95442, P=0.033). Meanwhile, we conducted a further analysis using Spearman correlation test, the consistent relationship between the expression of miR-542-5p and the clinicopathological parameters of HCC were listed as follows: tumor nodes (r=-0.278, P=0.005), metastasis (r=-0.221, P=0.026), TNM(-0.233, P=0.019), portal vein tumor embolus (r=-0.212, P=0.033) and tumor capsular infil-

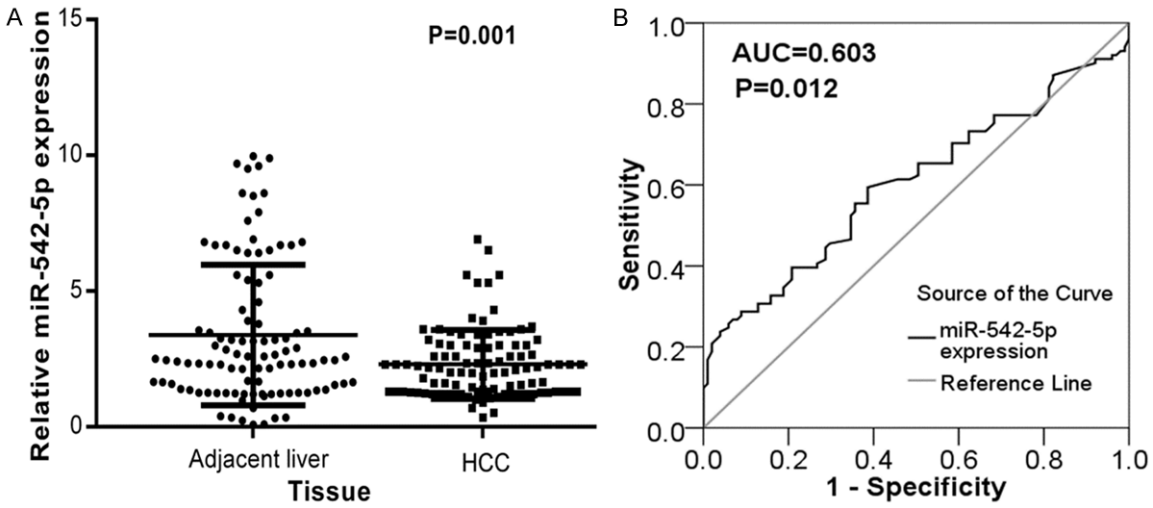

Figure 1. Expression of miR-542-5p in adjacent non-cancerous liver and HCC tissues. Quantitative real-time RT-PCR was performed to detect the expression of miR-542-5p. (A) The difference of relevant miR-542-5p expression between adjacent non-cancerous liver and HCC tissues. (B) ROC curve of miR-542-5p expression to distinguish HCC from non-cancerous liver. The area under curve (AUC) of miR-542-5p was 0.603 (95% CI: 0.524~0.681, *P*=0.012). Error bars represent standard deviation (SD). The statistical analysis was performed using paired Student's t-test (A and B).

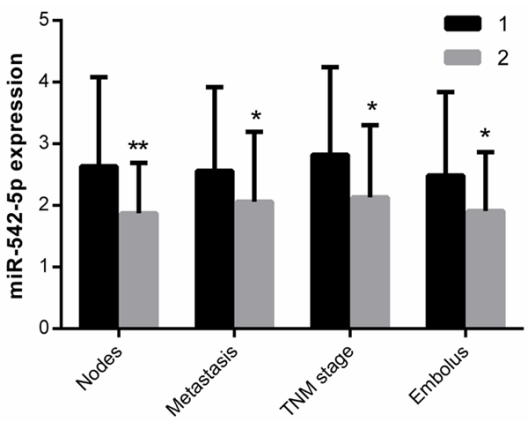

Figure 2. The relationship between miR-542-5p expression and clinicopathological parameters of HCC. A. Tumor nodes: 1. single tumor nodes; 2. multiple. B. Metastasis: 1. No; 2. Yes. C. Clinical TNM stage: 1. I-II; 2. III-IV. D. Portal vein tumor embolus: 1. No; 2. Yes. **P* < 0.05, ***P* < 0.01.

tration (r=-0.202, P=0.043). Additionally, the AUC of miR-542-5p for tumor nodes was 0.662 (95% CI: 0.556-0.767, P=0.005), for metastasis was 0.628 (95% CI: 0.519-0.736, P=0.027), for TNM was 0.656 (95% CI: 0.535-0.776, P=0.020), and for the portal vein tumor embolus was 0.631 (95% CI: 0.513-0.750, P=0.034) (**Figure 3**). However, no association was found between miR-542-5p expression and other clinicopathological features.

Relationship between EGFR and miR-542-5p expression

We initially compared the complementarity of miR-542-5p sequence to EGFR with Targetscan, miRTar and miRanda, respectively. Partial complementarity between miR-542-5p and EGFR was observed (data not shown). Furthermore, Spearman correlation test showed that EGFR expression was prominently negatively correlated with the miR-542-5p expression (r=-0.139, P=0.049). The expression of miR-542-5p was split into overexpression group and downexpression group by mean value. In miR-542-5p overexpression group, the expression of EGFR was down regulated by the rates of 31.9%, 30.6%, 22.2% and 15.3% corresponding to: -, 1+, 2+, 3+, respectively. While in miR-542-5p downexpression group, the expression rates of EGFR was 20.0%, 36.2%, 22.3% and 21.5% corresponding to: -, 1+, 2+, 3+, in order. When EGFR was divided into different grades for HCC and adjacent normal liver tissues to compare the expression of miR-542-5p, we found that expression of miR-542-5p in 3+ group was significantly lower than - and 1+ group (P=0.022 and P=0.037, respectively, **Figure 4**). The expressions of miR-542-5p of other groups showed no significant difference between each other.

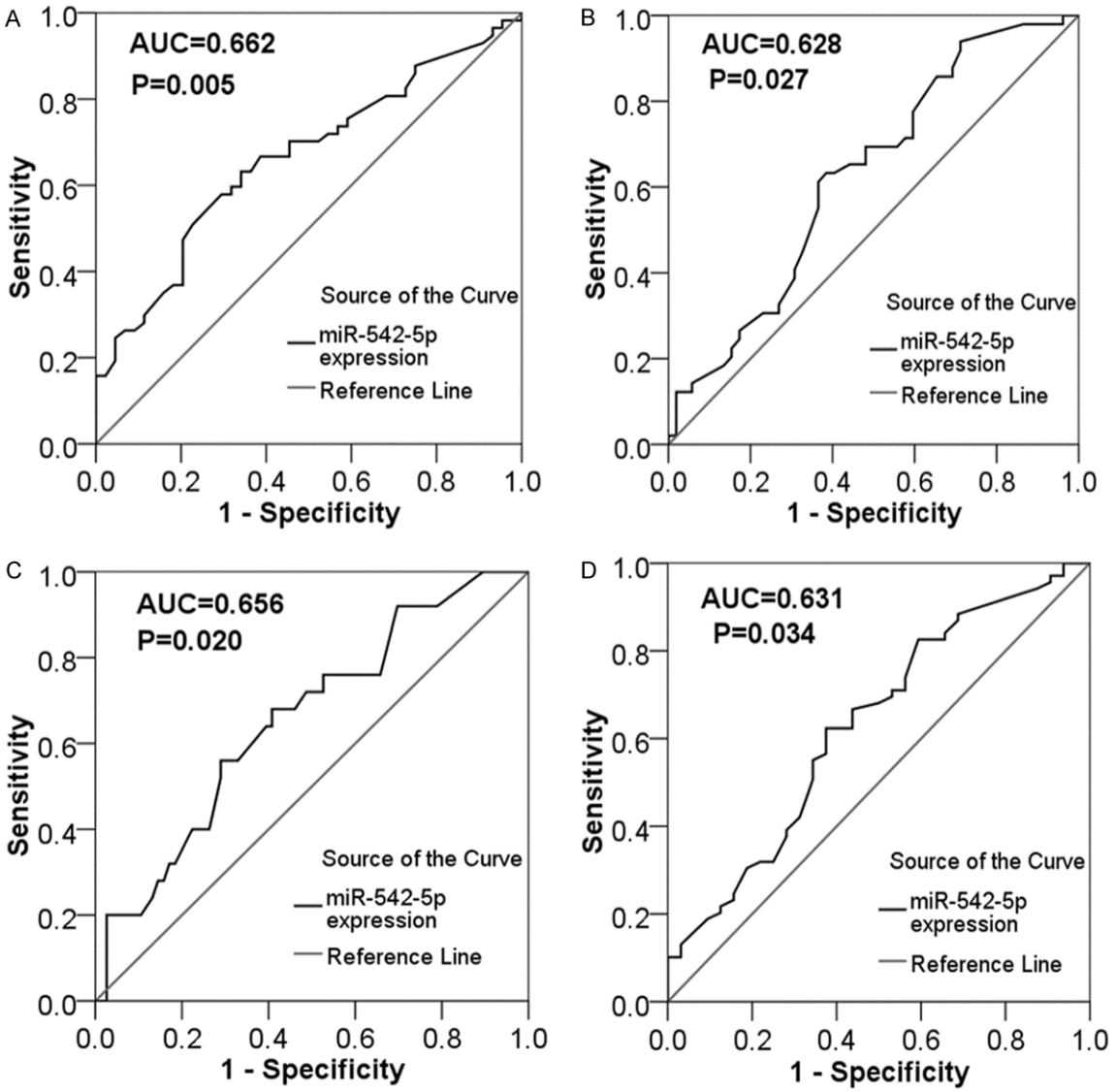

Figure 3. ROC curve of miR-542-5p expression of clinicopathological parameters. A. ROC curve of tumor nodes. The AUC was 0.662 (95% CI: 0.556-0.767, P=0.005). B. ROC curve of metastasis. The area under curve (AUC) was 0.628 (95% CI: 0.519~0.736, P=0.027). C. ROC curve of clinical TNM stage. The area under curve (AUC) was 0.656 (95% CI: 0.535~0.776, P=0.020). D. ROC curve of portal vein tumor embolus. The AUC was 0.631 (95% CI: 0.513~0.750, *P*=0.034).

Role of miR-542-5p expression in recurrence of HCC

Among all the 101 patients, we had a successful follow-up of 70 patients, among which 46 had low miR-542-5p expression (lower than the mean level of 2.3028), while 24 had high miR-542-5p expression. As for time to recurrence, low miR-542-5p expression group was 47.653±2.937 months, shorter than that of the high expression group (63.400±3.095 months). However, no significant difference of recurrent time was found between the low and high miR-542-5p groups (Chi-square=3.284, P=0.070) (**Figure 5**).

Discussion

As one of the most common malignancies worldwide, hepatic carcinoma is accounting for mortality mainly connected with malignant tumor. Many studies have been focused on the intervene of progression of hepatocarcinoma, but the five-year survival rate for patients with advanced hepatic carcinoma was still less than 5%, especially for patients with metastasis and

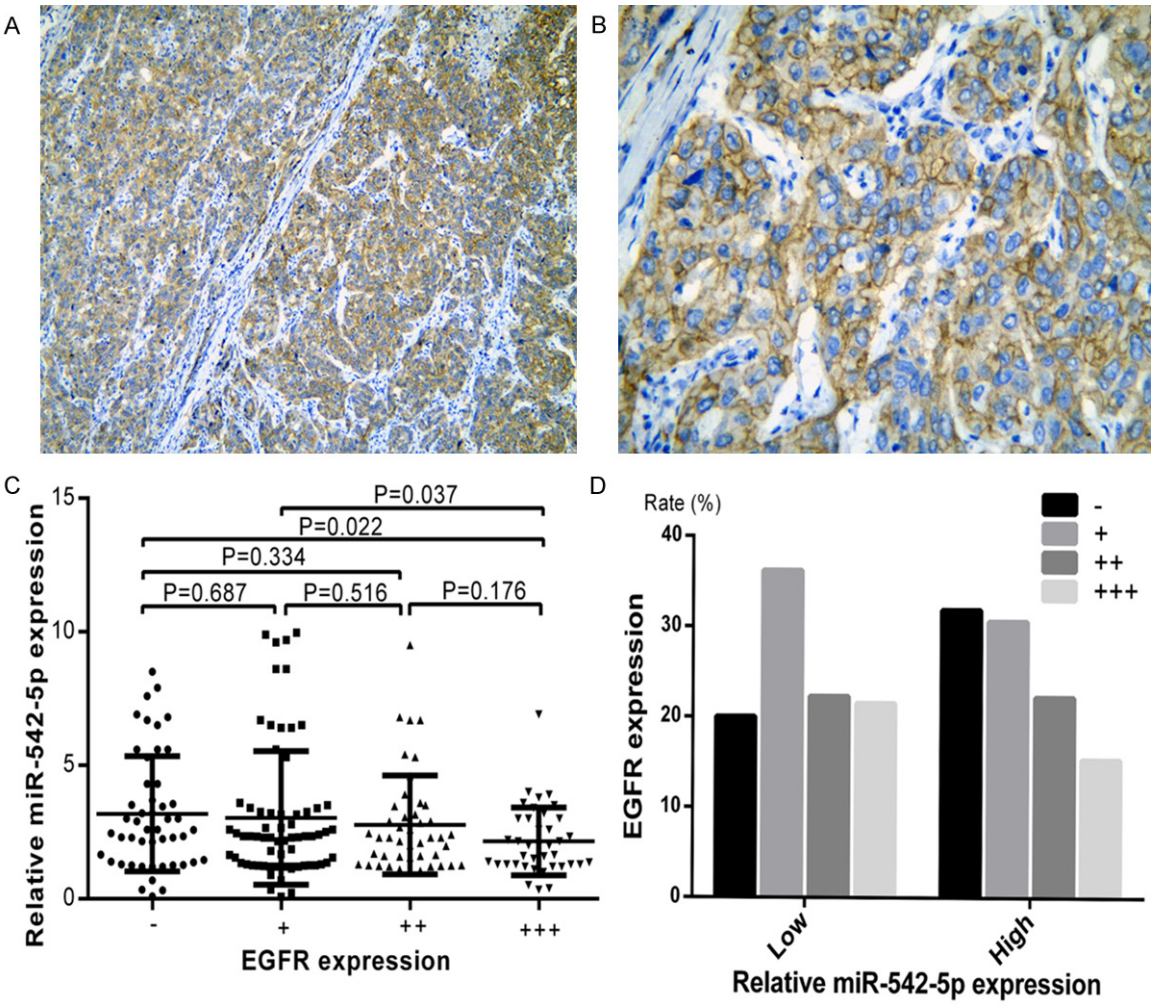

Figure 4. Relationship between EGFR and miR-542-5p expression. A. EGFR expression in HCC tissues (immunohistochemistry, ×100); B. EGFR expression in HCC tissues (immunohistochemistry, ×400); C. The expression of miR-542-5p in -, 1+, 2+, 3+ EGFR expression group; D. The expression of EGFR in downexpression group and overexpression group of miR-542-5p (The expression of miR-542-5p was split into overexpression group and downexpression group by mean value).

relapse, usually presenting poor prognosis [18]. Meanwhile, according to cancer statistics of 2015, it was indicated that thyroid cancer and liver cancer are the ones with fastest increasing prevalence rate [19]. Therefore, it is fairly urgent to seek better methods of diagnosis and therapy for early stage of liver cancer. With the development of molecular biological technique, quantities of biomarkers, which are intensively connected with metastasis, relapse and survival of HCC, have been excavated.

Growing evidence indicates that miRNAs exert important impacts on multiple biological processes. And the abnormal expression of miRNAs has been found in various human diseases. As a novel biomarker, miR-542-5p has been extensively studied in tumorigenesis, progression and metastasis in several types of cancer, such as neuroblastoma [20, 21], osteosarcoma [21], follicular carcinomas [23], endometrial carcinosarcomas [24], papillary thyroid carcinoma [25], basal cell carcinoma [26], lung cancer [27, 28], breast cancer [29] and rectal cancer [30]. It has been demonstrated that miR-542-5p reduced invasivity of neuroblastoma cells and was negatively correlated with poor prognosis of patients with neuroblastoma [20-22]. Katayama et al. [31] reported that miR-542-5p was relatively lowly expressed in 10 cases of normal liver tissue samples without virus infection, while highly expressed in surrounding non-tumor tissues of HCV-related HCC. But the study of miR-542-5p in HCC has

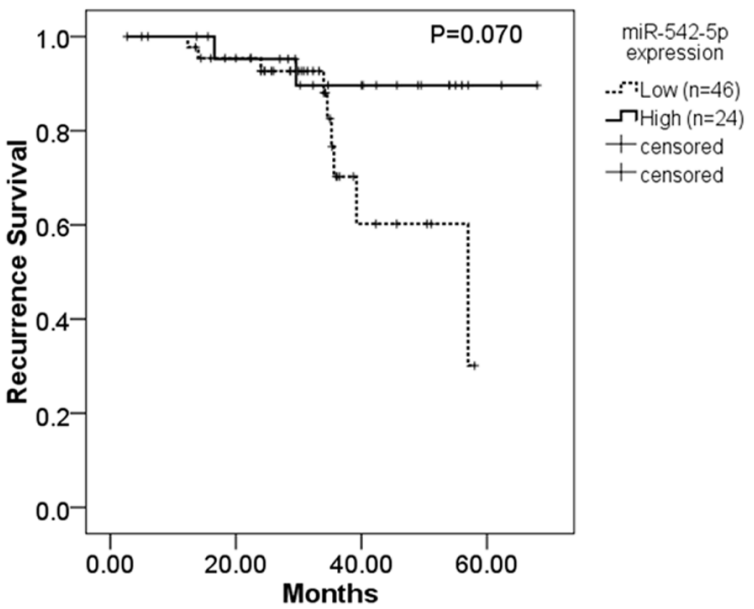

Figure 5. Role of miR-542-5p expression in recurrence of HCC. Kaplan-Meier survival curve showed that the recurrent time of low miR-542-5p group was shorter than high group, *P*=0.070.

not been fully established, the relationship between miR-542-5p and its corresponding clinicopathological parameters is still unknown, and whether it can be used as a biomarker for diagnosis of HCC is either unclear.

In this study, miR-542-5p was significantly expressed lower in 101 cases of HCC tissues, compared with adjacent noncancerous tissues (P=0.001). Besides, the expression of miR-542-5p was inversely correlated with clinical TNM stage, metastasis, tumor nodes, portal vein tumor embolus as well as tumor capsular infiltration (P < 0.05, respectively). The expression of miR-542-5p was significantly downregulated in patients with metastasis or in advance stage, indicating that miR-542-5p may exert tumor suppressive function through suppression of hepatocellular proliferation and invasion. When referred to relationship between miR-542-5p and HCV infection, we found that there existed inconspicuous upregulation of miR-542-5p in HCC tissues infected with HCV, which was inconsistent with the results of previous studies conducted by Katayama et al. [31]. HCV infection is likely to promote HCC occurrence. And Katayama et al. studied only a small patient size (n=12) with HCV infection, which may cause the difference between theirs and the current study. The high miR-542-5p expression group showed a longer recurrent time, though only a borderline difference of recurrent time was found between the low and high miR-542-5p groups (P=0.070). In addition, the diagnostic value of miR-542-5p in HCC was proved by ROC curve. The area under curve (AUC) of miR-542-5p for predicting advanced clinical stage (stage III/IV) was 0.656 (P= 0.020), suggesting its potential value as a biomarker for estimating poor prognosis in HCC.

Studies on mechanisms participated by miR-542-5p in oncogenesis has been carried out in recent years. Yamaguchi et al. [28] has demonstrated that EGFR mRNA is directly targeted by miR-542-5p in lung cancer cells. Joerger et al. [27] also reported that miR-542-5p can significantly predict outcome of patients with advanced NSCLC, and it down-regulated EGFR mRNA and protein expression. Further, Zhou et al. [32] reported that miR-542-5p could downregulate EGFRvA expression and inhibit migration and invasion of cancer cells. In addition, studies on cellular senescence program have also been reported. Faraonio et al. [33] demonstrated that miR-542-5p could induce double-strand DNA breaks and accumulation of reactive oxygen, cellular senescence, conferring cellular senescence program through the mTOR pathway in human diploid fibroblast. However, among other genes targeted by miR-542-5p, EGFR showed the strongest evidence in silico, which aroused our interests in mechanisms underlying these facts. Therefore, Spearman correlation test was performed to evaluate the EGFR expression and hint a negative correlation with the miR-542-5p expression. Later, we set up two groups based on miR-542-5p expression by mean value, and the results also indicated that the expression of EGFR was downregulated in miR-542-5p overexpression group. When divided EGFR into different grades to compare the expression of miR-542-5p, the results denoted that expression of miR-542-5p in 3+ group was significantly lower than - and 1+ group. The possible relationship still needs to be verified in vitro and in vivo.

Taken together, our findings demonstrated that miR-542-5p was lower expressed in patients with HCC, possibly via targeting EGFR mRNA. MiR-542-5p may serve as an assistant biomarker for the diagnosis and prognosis of HCC. Further investigations are desired to explore the mechanism underlying the effect of miR-542-5p on tumor metastasis and invasion.

Acknowledgements

The study was supported partly by the Fund of Future Academic Star Project of Guangxi Medical University (WLXSZX1547), Guangxi Provincial Health Bureau Scientific Research Project (Z2014054), Youth Science Foundation of Guangxi Medical University (GXMUYSF20-1311), Guangxi University Science and Technology Research Projects (LX2014075), and the Fund of National Natural Science Foundation of China (NSFC 81360327, NSFC8156-0489). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the-manuscript.

Disclosure of conflict of interest

None.

Authors' contributions

LJJ conceived of the study and participated in its design and coordination. YX and DYW performed the main part of the experiments and drafted the manuscript. LYH, WHL, LFF, WX, LXM, HRQ and LXG cooperated the experiments, drafted the manuscript and performed the statistical analyses. CG designed the study, supervised the experiments and corrected the paper. All authors read and approved the final manuscript.

Address correspondence to: Drs. Yi-Wu Dang and Gang Chen, Department of Pathology, First Affiliated Hospital of Guangxi Medical University, 6 Shuangyong Road, Nanning 530021, Guangxi Zhuang Autonomous Region, P. R. China. Fax: +86 771 535-8943; E-mail: dangyiwu@126.com (YWD); chen_gang_triones@163.com (GC)

References

[1] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-2917.

[2] Jemal A, Center MM, DeSantis C, Ward EM. Global patterns of cancer incidence and mortality rates and trends. Cancer Epidemiol Biomarkers Prev 2010; 19: 1893-1907.

[3] McGlynn KA, Tsao L, Hsing AW, Devesa SS, Fraumeni JF Jr. International trends and patterns of primary liver cancer. Int J Cancer 2001; 94: 290-296.

[4] Fares N, Peron JM. Epidemiology, natural history, and risk factors of hepatocellular carcinoma. Rev Prat 2013; 63: 216-217, 220-212.

[5] La Vecchia C, Lucchini F, Franceschi S, Negri E, Levi F. Trends in mortality from primary liver cancer in Europe. Eur J Cancer 2000; 36: 909-915.

[6] Callegari E, Elamin BK, Sabbioni S, Gramantieri L, Negrini M. Role of microRNAs in hepatocellular carcinoma: a clinical perspective. Onco Targets Ther 2013; 6: 1167-1178.

[7] Bray I, Tivnan A, Bryan K, Foley NH, Watters KM, Tracey L, Davidoff AM, Stallings RL. MicroRNA-542-5p as a novel tumor suppressor in neuroblastoma. Cancer Lett 2011; 303: 56-64.

[8] Schulte JH, Schowe B, Mestdagh P, Kaderali L, Kalaghatgi P, Schlierf S, Vermeulen J, Brockmeyer B, Pajtler K, Thor T, de Preter K, Speleman F, Morik K, Eggert A, Vandesompele J, Schramm A. Accurate prediction of neuroblastoma outcome based on miRNA expression profiles. Int J Cancer 2010; 127: 2374-2385.

[9] Mestdagh P, Fredlund E, Pattyn F, Schulte JH, Muth D, Vermeulen J, Kumps C, Schlierf S, De Preter K, Van Roy N, Noguera R, Laureys G, Schramm A, Eggert A, Westermann F, Speleman F, Vandesompele J. MYCN/c-MYC-induced microRNAs repress coding gene networks associated with poor outcome in MYCN/c-MYC-activated tumors. Oncogene 2010; 29: 1394-1404.

[10] Althoff K, Lindner S, Odersky A, Mestdagh P, Beckers A, Karczewski S, Molenaar JJ, Bohrer A, Knauer S, Speleman F, Epple M, Kozlova D, Yoon S, Baek K, Vandesompele J, Eggert A, Schramm A, Schulte JH. miR-542-3p exerts tumor suppressive functions in neuroblastoma by downregulating Survivin. Int J Cancer 2015; 136: 1308-20.

[11] Budhu A, Jia HL, Forgues M, Liu CG, Goldstein D, Lam A, Zanetti KA, Ye QH, Qin LX, Croce CM, Tang ZY, Wang XW. Identification of metastasis-related microRNAs in hepatocellular carcinoma. Hepatology 2008; 47: 897-907.

[12] Yamaguchi G, Takanashi M, Tanaka M. Fujita K, Ohira T, Kuroda M, Ikeda N. Isolation of miRNAs that target EGFR mRNA in human lung cancer. Biochem Biophys Res Commun 2012; 420: 411-6.

[13] Rong MH, He RQ, Dang YW, Chen G. Expression and clinicopathological significance of miR-146a in hepatocellular carcinoma tissues. Ups J Med Sci 2014; 119: 19-24.

[14] Rong MH, Chen G, Dang YW. Increased MiR-221 expression in hepatocellular carcinoma tissues and its role in enhancing cell growth and inhibiting apoptosis in vitro. BMC Cancer 2013; 13: 21.
[15] Dang YW, Luo DZ, Rong MH, Chen G. Underexpression of miR-34a in Hepatocellular Carcinoma and Its Contribution towards Enhancement of Proliferating Inhibitory Effects of Agents Targeting c-MET. PLoS One 2013; 8: e61054.
[16] Chen G, Umelo IA, Lv SS, Teugels E, Fostier K, Kronenberger P, Dewaele A, Sadones J, Geers C, De Grève J. miR-146a Inhibits Cell Growth, Cell Migration and Induces Apoptosis in Non-Small Cell Lung Cancer Cells. PLoS One 2013; 8: e60317.
[17] Chen G, Kronenberger P, Teugels E, Umelo IA, De Grève J. Targeting the epidermal growth factor receptor in non-small cell lung cancer cells: the effect of combining RNA interference with tyrosine kinase inhibitors or cetuximab. BMC Med 2012; 10: 28.
[18] Zhang Z, Zhang Y, Sun XX, Ma X, Chen ZN. microRNA-146a inhibits cancer metastasis by downregulating VEGF through dual pathways in hepatocellular carcinoma. Mol Cancer 2015; 14: 5.
[19] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5-29.
[20] Althoff K, Lindner S, Odersky A, Mestdagh P, Beckers A, Karczewski S, Molenaar JJ, Bohrer A, Knauer S, Speleman F, Epple M, Kozlova D, Yoon S, Baek K, Vandesompele J, Eggert A, Schramm A, Schulte JH. miR-542-3p exerts tumor suppressive functions in neuroblastoma by downregulating Survivin. Int J Cancer 2015; 136: 1308-1320.
[21] Mosakhani N, Pazzaglia L, Benassi M S, Borze I, Quattrini I, Picci P, Knuutila S. MicroRNA expression profiles in metastatic and non-metastatic giant cell tumor of bone. Histol Histopathol 2013; 28: 671-678.
[22] Lulla RR, Costa FF, Bischof JM, Chou PM, de F Bonaldo M, Vanin EF, Soares MB. Identification of Differentially Expressed MicroRNAs in Osteosarcoma. Sarcoma 2011; 732690.
[23] Dettmer M, Vogetseder A, Durso MB, Moch H, Komminoth P, Perren A, Nikiforov YE, Nikiforova MN. MicroRNA expression array identifies novel diagnostic markers for conventional and oncocytic follicular thyroid carcinomas. J Clin Endocrinol Metab 2013; 98: E1-7.
[24] Castilla MA, Moreno-Bueno G, Romero-Perez L, Van De Vijver K, Biscuola M, López-García MÁ, Prat J, Matías-Guiu X, Cano A, Oliva E, Palacios J. Micro-RNA signature of the epithelial-mesenchymal transition in endometrial carcinosarcoma. J Pathol 2011; 223: 72-80.
[25] Wang Z, Zhang H, Zhang P, Li J, Shan Z, Teng W. Upregulation of miR-2861 and miR-451 expression in papillary thyroid carcinoma with lymph node metastasis. Med Oncol 2013; 30: 577.
[26] Sand M, Skrygan M, Sand D, Georgas D, Hahn SA, Gambichler T, Altmeyer P, Bechara FG. Expression of microRNAs in basal cell carcinoma. Br J Dermatol 2012; 167: 847-855.
[27] Joerger M, Baty F, Fruh M, Droege C, Stahel RA, Betticher DC, von Moos R, Ochsenbein A, Pless M, Gautschi O, Rothschild S, Brauchli P, Klingbiel D, Zappa F, Brutsche M. Circulating microRNA profiling in patients with advanced non-squamous NSCLC receiving bevacizumab/erlotinib followed by platinum-based chemotherapy at progression (SAKK 19/05). Lung Cancer 2014; 85: 306-313.
[28] Yamaguchi G, Takanashi M, Tanaka M, Fujita K, Ohira T, Kuroda M, Ikeda N. Isolation of miRNAs that target EGFR mRNA in human lung cancer. Biochem Biophys Res Commun 2012; 420: 411-416.
[29] Wang B, Li J, Sun M, Sun L, Zhang X. miRNA expression in breast cancer varies with lymph node metastasis and other clinicopathologic features. IUBMB Life 2014; 66: 371-377.
[30] Gaedcke J, Grade M, Camps J, Søkilde R, Kaczkowski B, Schetter AJ, Difilippantonio MJ, Harris CC, Ghadimi BM, Møller S, Beissbarth T, Ried T, Litman T. The rectal cancer microRNAome--microRNA expression in rectal cancer and matched normal mucosa. Clin Cancer Res 2012; 18: 4919-4930.
[31] Katayama Y, Maeda M, Miyaguchi K, Nemoto S, Yasen M, Tanaka S, Mizushima H, Fukuoka Y, Arii S, Tanaka H. Identification of pathogenesis-related microRNAs in hepatocellular carcinoma by expression profiling. Oncol Lett 2012; 4: 817-823.
[32] Zhou M, Wang H, Zhou K, Luo X, Pan X, Shi B, Jiang H, Zhang J, Li K, Wang HM, Gao H, Lu S, Yao M, Mao Y, Wang HY, Yang S, Gu J, Li C, Li Z. A novel EGFR isoform confers increased invasiveness to cancer cells. Cancer Res 2013; 73: 7056-7067.
[33] Faraonio R, Salerno P, Passaro F, Sedia C, Iaccio A, Bellelli R, Nappi TC, Comegna M, Romano S, Salvatore G, Santoro M, Cimino F. A set of miRNAs participates in the cellular senescence program in human diploid fibroblasts. Cell Death Differ 2012; 19: 713-721.