A *miR-182* variant and risk of hepatocellular carcinoma in a southern Chinese population

Moqin Qiu[1,2], Yingchun Liu[1,3], Qiuling Lin[1,2], Zihan Zhou[1,2], Yanji Jiang[1,2], Rongrui Huo[1], Xiumei Liang[1], Xiangyuan Yu[4,5], Ji Cao[1], Xianguo Zhou[1,3*] and Hongping Yu[1,2,3*]

Abstract

MicroRNAs (miRNAs) play important roles in the regulation of gene expression at the posttranscriptional level and are involved in human carcinogenesis. The aim of the current study was to investigate the associations between *miR-182* single nucleotide polymorphisms and HCC risk in a southern Chinese population. In this case-control study of 863 HCC patients and 908 cancer-free controls, we performed genotyping of *miR-182* rs4541843 and assessed its association with HCC risk. We found that individuals carrying the AG/AA genotypes of *miR-182* rs4541843 were significantly associated with an increased risk of HCC compared with those carrying the GG genotype (adjusted odds ratio (OR) = 1.71, 95% confidence interval (CI) = 1.07–2.76, P = 0.026). In the stratified analysis, this increased risk was more pronounced in the subgroups of older individuals (adjusted OR = 1.98, 95% CI = 1.04–3.76, P = 0.037), males (adjusted OR = 1.81, 95% CI = 1.09–2.99, P = 0.021), and never drinkers (adjusted OR = 1.84, 95% CI = 1.03–3.30, P = 0.041). Our results suggested that *miR-182* polymorphism rs4541843 may contribute to the susceptibility to HCC. Our findings require validation in further studies with larger sample sizes.

Keywords: Hepatocellular carcinoma, *miR-182*, Polymorphism, Risk, Association

Introduction

The incidence of liver cancer in China is markedly higher than that in other geographical areas around the world [1]. In 2018, approximately 841,000 people worldwide were diagnosed with liver cancer, and approximately half of these diagnoses were in China [2]. The major histological subtype of liver cancer is hepatocellular carcinoma (HCC), accounting for approximately 75–80% of all cases [3]. Several key factors may contribute to HCC, such as hepatitis B/C virus (HBV/HCV) infection, cigarette smoking, and heavy drinking, but only a small fraction of exposed individuals eventually develop HCC in their lifetimes [4]. Recently, several genome-wide association studies have identified susceptible loci harboring common single nucleotide polymorphisms

(SNPs) that are relevant to the risk of HCC, suggesting that genetic factors may be responsible for susceptibility to HCC [5–8].

MicroRNAs (miRNAs) are a class of small non-coding RNAs that can regulate gene expression by pairing with the 3′ untranslated region (3′ UTR) of mRNAs [9]. To date, more than 1800 precursor miRNAs have been found in the human genome, and aberrant expression of miR-NAs has been related to the etiology, diagnosis, and prognosis of many human cancers including HCC [10–12]. *MiR-182*, located in a 5-kb region of human chromosome 7q32.2, is a member of the miR-183/96/182 cluster [13]. Recently, *miR-182* has emerged as a high-priority miRNA in HCC and is involved in the development of HCC by regulating various biological processes, including cell growth, differentiation, migration, invasion, and apoptosis [14–16]. MiR-182 is frequently expressed at high levels in

* Correspondence: zhouxianguo1001@163.com; yhp268@163.com
[1]Guangxi Medical University Cancer Hospital, Nanning 530021, China
Full list of author information is available at the end of the article

Qiu *et al. Human Genomics*   (2020) 14:38

Page 2 of 5

HCC, and its high expression is correlated with the metastasis, recurrence, and poor prognosis of HCC [17, 18].

Emerging studies have shown that SNPs in miRNAs may alter mRNA expression of their own or target genes, thus leading to tumorigenesis [19–21]. To our knowledge, there are no studies assessing the associations between *miR-182* SNPs and the risk of HCC. Given the important roles of *miR-182* in tumorigenesis, it is important to determine whether *miR-182* SNPs contribute to the susceptibility to HCC. In the current study, we conducted genotyping of *miR-182* polymorphisms and assessed their associations with HCC risk in a southern Chinese population.

Materials and methods
Study population
The study population consisted of 863 HCC patients who had undergone surgery between August 2007 and November 2011 in the First Affiliated Hospital of Guangxi Medical University, Affiliated Tumor Hospital of Guangxi Medical University, and First Affiliated Hospital of Guilin Medical University. The tumors were histopathologically confirmed independently as HCC by two pathologists as routine diagnosis. Patients with a prior history of other cancers, metastasized cancers, or previous radiotherapy or chemotherapy before recruitment were excluded. The 908 cancer-free controls were recruited at the same period in Southern China and were genetically unrelated and frequency matched to the cases by age (± 5 years) and sex. Participants with a history of cancer were excluded. The research protocol was approved by the Ethical Committee Review Board of Guangxi Medical University and Guilin Medical University.

With a signed written informed consent form during the interview, each subject enrolled in the study was interviewed to gather the information-related demographic data, history of tobacco and alcohol consumption, and chronic HBV infection through face-to-face interviews conducted by trained investigators. Ever smokers were defined as subjects who had smoked more than 100 cigarettes in their lifetimes; ever drinkers were defined as subjects who had used alcohol at least once a week for more than 1 year. HBV infection was defined as positive for HBV surface antigen (HBsAg). Of the 863 HCC patients, 370 HCC patients had long-term follow-up data for survival analysis. Clinical information (including AFP level, BCLC stage, cancer embolus, and cirrhosis) was collected from medical records. At 3-month intervals, follow-up information on deaths was updated by a trained clinical specialist via telephone. The survival time was calculated in months from the date of tumor resection to the date of death or last follow-up. The patients were followed up until 24 February 2020. A 5-ml peripheral blood sample was obtained from each study subject, of which 1 ml was used to determine the HBV infection status.

SNPs selection and genotyping
The selected SNPs were screened and identified by NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/) and Haploreg v4.1 (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) in accordance with three criteria: (1) location at the *miR-182* gene region; (2) minor allele frequency (MAF) in Chinese Han population > 0.05; and (3) low linkage disequilibrium using an r^2 threshold of < 0.8 for each. Ultimately, only one SNP (rs4541843 in *miR-182*) was selected in this study. Genomic DNA was extracted from peripheral blood by phenol-chloroform extraction and stored at − 80 ℃. The selected SNP was genotyped using the Agena MassARRAY genotyping system (Agena, San Diego, CA) according to the manufacturer's instructions. To ensure quality control, 10% of the samples were randomly selected to repeat the genotyping assays, and the reproducibility was 100%.

Statistical analysis
Differences in the distributions of demographic characteristics, selected variables and frequencies of genotypes of *miR-182* rs4541843 between the cases and controls were evaluated using the χ^2 test. Deviation of genotype frequencies in controls was tested with goodness-of-fit χ^2 tests for Hardy-Weinberg equilibrium (HWE). We estimated the associations between *miR-182* rs4541843 genotypes and HCC risk by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) with an unconditional multivariate logistic regression model. The associations between rs4541843 genotypes and HCC risk were also stratified by age, sex, smoking, drinking, and HBV infection. Hazard ratios (HRs) and 95% CIs were estimated by multivariate Cox proportional hazards model. We performed expression quantitative trait loci (eQTL) analyses with the genomic data from the genotype-tissue expression (GTEx) project to identify correlations between *miR-182* rs4541843 and mRNA expression levels of *miR-182*'s target genes. Two-sided tests of statistical significance were conducted using SPSS software, version 18.0 (SPSS Institute, Chicago, IL), and the result was considered significant when $P < 0.05$ by two-sided tests.

Results
Characteristics of the study population
The details of 863 HCC cases and 908 controls enrolled in this study are shown in Table 1. Because of frequency matching by design, there were no significant differences in the distributions of age or sex between the cases and controls ($P = 0.687$ and 0.053, respectively). However, cases were more likely to be smokers (43.0% vs. 25.2%, $P < 0.001$) and drinkers (38.5 vs. 21.7, $P < 0.001$) than the

Qiu *et al. Human Genomics*   (2020) 14:38

Page 3 of 5

Table 1 Frequency distributions of selected variables in HCC cases and controls

Variables	Cases n(%)	Controls n(%)	P [a]
All subjects	863(100)	908(100)	
Age			0.687
≤ 49	467(54.1)	500(55.1)	
> 49	396(45.9)	408(44.9)	
Sex			0.053
Females	100(11.6)	80(8.8)	
Males	763(88.4)	828(91.2)	
Smoking			< 0.001
Never	492(57.0)	679(74.8)	
Ever	371(43.0)	229(25.2)	
Drinking			< 0.001
Never	531(61.5)	711(78.3)	
Ever	332(38.5)	197(21.7)	
HBV infection			< 0.001
(−)	138(16.0)	821(90.4)	
(+)	725(84.0)	87(9.6)	

[a] Two sides chi-square test

controls. In addition, the percentage of subjects that responded positively to HBV infection was significantly higher among the HCC cases than among the controls (84.0% vs. 9.6%, $P < 0.001$). Therefore, these variables were further adjusted in later multivariate logistic regression analyses for any residual confounding effect.

Associations of *miR-182* rs4541843 with the risk of HCC

The observed genotype frequencies for this SNP among the control subjects were in agreement with HWE ($P = 0.625$ for *miR-182* rs4541843). After adjusted for age, gender, smoking status, drinking status, and HBV infection status, we found that the AG/AA genotypes for rs4541843 were significantly associated with an increased risk of HCC compared with the GG genotype (AG/AA vs. GG: adjusted OR = 1.71, 95% CI = 1.07–2.76, $P = 0.026$) (Table 2).

To further identify the relationship between *miR-182* rs4541843 and HCC risk, the dominant genetic model of

Table 2 Genotype frequencies of rs4541843 in cases and controls and their associations with HCC risk

Variants	Cases	Controls	P [a]	Adjust OR (95% CI)	P [b]
rs4541843					
GG	771	833	0.222	1	
AG	91	74		**1.71(1.06–2.74)**	**0.027**
AA	1	1		1.81(0.02–139.1)	0.789
AG/AA	92	75	0.084	**1.71(1.07–2.76)**	**0.026**

[a]Two sides chi-square test
[b]Adjusted for age, sex, smoking, drinking, and HBV infection in logistic regression models

this SNP was stratified by subgroups of age, sex, smoking, drinking, and HBV infection. As shown in Table 3, we found that the effect of *miR-182* rs4541843 on HCC risk was more pronounced in the older group (adjusted OR = 1.98, 95% CI = 1.04–3.76, $P = 0.037$), males (adjusted OR = 1.81, 95% CI = 1.09–2.99, $P = 0.021$), and never-drinking group (adjusted OR = 1.84, 95% CI = 1.03–3.30, $P = 0.041$), while no significant interaction between *miR-182* rs4541843 genotypes and environmental factors was observed ($P > 0.05$).

Association between *miR-182* rs4541843 and prognosis of patients with HCC

We investigated the associations between SNP rs4541843 and the clinical features of 370 HCC patients, such as AFP level, BCLC stage, cancer embolus, and cirrhosis, but failed to find any significant associations (Table 1S). We performed a multivariate cox regression analysis on the survival of HCC patients, and the result showed that the survival of HCC patients was related to BCLC stage and cancer embolus, but not rs4541843 (Table 2S).

Correlation between rs4541843 genotypes and mRNA expression levels of miR-182' s target genes

Previous studies have confirmed that *miR-182* could promote HCC progression by targeting multiple genes, such as *MTSS1*, *TP53INP1*, and *RASA1* [18, 22, 23]. So, we conducted the eQTL analysis to evaluate the correlations between SNP rs4541843 genotypes and the expression levels of these genes using the data of the GTEx

Table 3 Stratified analysis between rs4541843 polymorphism and HCC risk

variables	rs4541843 (cases/controls)		Adjust OR (95% CI)	P [a]
	GG	AG/AA	AG/AA vs. GG	
Age				
≤ 49	418/455	49/45	1.44(0.71–2.91)	0.310
> 49	353/378	43/30	**1.98(1.04–3.76)**	**0.037**
Sex				
Females	90/72	10/8	1.19(0.30–4.63)	0.807
Males	681/761	82/67	**1.81(1.09–2.99)**	**0.021**
Smoking				
Never	432/624	60/55	1.79(0.99–3.24)	0.055
Ever	339/209	32/20	1.62(0.73–3.60)	0.235
Drinking				
Never	468/655	63/56	**1.84(1.03–3.30)**	**0.041**
Ever	303/178	29/19	1.51(0.67–3.40)	0.326
HBV infection				
(−)	119/752	19/69	1.73(0.98–3.05)	0.060
(+)	652/81	73/6	1.62(0.68–3.86)	0.277

[a]Adjusted for age, sex, smoking, drinking, and HBV infection in logistic regression models

database. Although we found that the A allele of SNP rs4541843 appeared to be correlated with the decreased expression levels of MTSS1, TP53INP1, and RASA1 in the liver tissues, the correlations between them did not reach statistically significant level (Supplementary Figure 1).

Discussion

In the present study, we investigated whether miR-182 polymorphisms were associated with the risk of HCC in a southern Chinese population, and found that miR-182 rs4541843 AG/AA genotypes were significantly associated with an evidently increased HCC risk, especially in older group, males and never-drinking group. Our findings suggest, for the first time, that genetic variant of miR-182 might contribute to the development of HCC in this population.

MiR-182 has been reported to act as a tumor promoter in HCC, and its upregulation has been shown to be associated with the development, metastasis, recurrence, and poor prognosis of HCC. For instance, aberrant miR-182 expression can promote HCC metastasis by targeting metastasis suppressor 1 (MTSS1), and miR-182 may be used as a diagnostic marker or potential therapeutic target in HCC [18]. Cao et al. investigated the function of miR-182-5p and found that the over-expression of miR-182-5p was related to poor prognosis and early recurrence in HCC. Subsequent functional experiments showed that miR-182-5p could repress FOXO3a expression by directly targeting its 3′UTR, activating the AKT/FOXO3a pathway to promote HCC progression [22]. Du et al. found that miR-182 was induced in HCC cells under hypoxia and promoted angiogenesis by targeting RASA1 [24]. Additionally, miR-182 plays an important role in drug resistance and upregulated miR-182-induced cisplatin resistance in HCC cells by regulating tumor protein 53-induced nuclearprotein 1 (TP53INP1) [23]. So, we conducted the eQTL analysis to evaluate the correlations between SNP rs4541843 genotypes and the mRNA expression levels of miR-182's target genes using the data of the GTEx database. Although we found that the A allele of the SNP rs4541843 appeared to be correlated with the decreased expression levels of MTSS1, TP53INP1, and RASA1 in the liver tissues, the correlations between them did not reach statistically significant level. Tumorigenesis is a complex process, it is reasonable to speculate that miR-182 rs4541843 might simultaneously alter the expression of multiple target genes, and subsequently contribute to the risk of HCC. However, further functional studies and association studies with larger sample sizes are warranted to confirm our findings.

Several studies have reported that single nucleotide polymorphisms in miRNA genes may influence individual susceptibility to cancers including HCC [25–27]. Xu et al. investigated the influence of miR-146a rs2910164

(G > C) on individual susceptibility to HCC and found that males with the GG genotype of miR-146a gene were 2-fold more susceptible to HCC than those carrying the CC genotype (OR = 2.02, 95% CI = 1.06–3.85, P = 0.034) [28]. Further investigation revealed that the GG genotype conferred a higher expression level of mature miR-146a, therefore was more susceptible to carcinogens. Liu et al. showed that the A-to-G base change in rs999885 in the promoter region of the miR-106b-25 cluster may lead to an increased risk for HCC in HBV-persistent carriers by altering the expression of the miR-106b-25 cluster [29]. Bei et al. showed that SNP of rs1135519 could modulate miR-122 expression and contribute to the genetic susceptibility of HCC, either independently or together with rs9966765 in miR-122 [30]. In our study, we selected one common SNP located in miR-182 and found that the miR-182 rs4541843 AG/AA genotypes were associated with a significantly increased risk of HCC. In the stratification analysis by variables, we observed that this significant association with the risk of HCC was particularly pronounced in the older group, males and the never-drinking group. However, studies of larger sample sizes among different populations are needed to confirm our findings.

Some potential limitations of our study should be considered. First, because our study was a hospital-based case-control study with an unavoidable selection bias, we applied a rigorous design in selecting study subjects, and controls were well matched to cases according to age and sex to minimize potential biases. Second, although the sample size of our study was large, a relatively small size in some subgroup analyses may have limited the statistical power. Finally, the precise molecular mechanism underlying the observed effects require further investigation.

In conclusion, our study provides evidence supporting the notion that the miR-182 polymorphism might be associated with HCC risk in a southern Chinese population. Further studies with larger sample sizes and functional studies are warranted to validate our findings.

Supplementary information

Additional file 1: Table 1S. The associations between miR-182 rs4541843 polymorphism and clinical features of HCC patients. **Table 2S.** Cox regression analysis of the prognosis of HCC Patients.

Additional file 2: Figure S1. The correlation between miR-182 rs4541843 and mRNA expression of its target genes in the liver tissues from the genotype-tissue expression database.

Qiu *et al. Human Genomics*   (2020) 14:38

Page 5 of 5

Author details
[1]Guangxi Medical University Cancer Hospital, Nanning 530021, China. [2]School of Public Health, Guangxi Medical University, Nanning 530021, China. [3]Guangxi Cancer Molecular Medical Engineering Research Center, Nanning 530021, China. [4]Affiliated Hospital of Guilin Medical University, Guilin 541004, China. [5]School of Public Health, Guilin Medical University, Guilin 541004, China.

References
1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65:87–108.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.
3. Villanueva A. Hepatocellular Carcinoma. N Engl J Med. 2019;380:1450–62.
4. Lafaro KJ, Demirjian AN, Pawlik TM. Epidemiology of hepatocellular carcinoma. Surg Oncol Clin N Am. 2015;24:1–17.
5. Li Y, Zhai Y, Song Q, Zhang H, Cao P, Ping J, et al. Genome-Wide Association Study Identifies a New Locus at 7q21.13 Associated with Hepatitis B Virus-Related Hepatocellular Carcinoma. Clin Cancer Res. 2018;24: 906–15.
6. Zhang H, Zhai Y, Hu Z, Wu C, Qian J, Jia W, et al. Genome-wide association study identifies 1p36.22 as a new susceptibility locus for hepatocellular carcinoma in chronic hepatitis B virus carriers. Nat Genet. 2010;42:755–8.
7. Matsuura K, Sawai H, Ikeo K, Ogawa S, Iio E, Isogawa M, et al. Genome-Wide Association Study Identifies TLL1 Variant Associated With Development of Hepatocellular Carcinoma After Eradication of Hepatitis C Virus Infection. Gastroenterology. 2017;152:1383–94.
8. Lee MH, Huang YH, Chen HY, Khor SS, Chang YH, Lin YJ, et al. Human leukocyte antigen variants and risk of hepatocellular carcinoma modified by hepatitis C virus genotypes: A genome-wide association study. Hepatology. 2018;67:651–61.
9. Ambros V. The functions of animal microRNAs. Nature. 2004;431:350–5.
10. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116:281–97.
11. Xu J, Zhu X, Wu L, Yang R, Yang Z, Wang Q, et al. MicroRNA-122 suppresses cell proliferation and induces cell apoptosis in hepatocellular carcinoma by directly targeting Wnt/beta-catenin pathway. Liver Int. 2012;32:752–60.
12. Ura S, Honda M, Yamashita T, Ueda T, Takatori H, Nishino R, et al. Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma. Hepatology. 2009;49:1098–112.
13. Woldemichael BT, Jawaid A, Kremer EA, Gaur N, Krol J, Marchais A, et al. The microRNA cluster miR-183/96/182 contributes to long-term memory in a protein phosphatase 1-dependent manner. Nature Communications. 2016;7:12594.
14. Li P, Sheng C, Huang L, Zhang H, Huang L, Cheng Z, et al. MiR-183/-96/-182 cluster is up-regulated in most breast cancers and increases cell proliferation and migration. Breast Cancer Res. 2014;16:473.
15. Segura MF, Hanniford D, Menendez S, Reavie L, Zou X, Alvarez-Diaz S, et al. Aberrant miR-182 expression promotes melanoma metastasis by repressing FOXO3 and microphthalmia-associated transcription factor. Proc Natl Acad Sci U S A. 2009;106:1814–9.
16. Wang X, Li H, Cui L, Feng J, Fan Q. MicroRNA-182 suppresses clear cell renal cell carcinoma migration and invasion by targeting IGF1R. Neoplasma. 2016; 63:717–25.
17. Chen L, Chu F, Cao Y, Shao J, Wang F. Serum miR-182 and miR-331-3p as diagnostic and prognostic markers in patients with hepatocellular carcinoma. Tumour Biol. 2015;36:7439–47.
18. Wang J, Li J, Shen J, Wang C, Yang L, Zhang X. MicroRNA-182 downregulates metastasis suppressor 1 and contributes to metastasis of hepatocellular carcinoma. BMC cancer. 2012;12:227.
19. Shi TY, Chen XJ, Zhu ML, Wang MY, He J, Yu KD, et al. A pri-miR-218 variant and risk of cervical carcinoma in Chinese women. BMC Cancer. 2013;13:19.
20. Xu Y, Liu L, Liu J, Zhang Y, Zhu J, Chen J, et al. A potentially functional polymorphism in the promoter region of miR-34b/c is associated with an increased risk for primary hepatocellular carcinoma. Int J Cancer. 2011;128:412–7.
21. Xie K, Liu J, Zhu L, Liu Y, Pan Y, Wen J, et al. A potentially functional polymorphism in the promoter region of let-7 family is associated with survival of hepatocellular carcinoma. Cancer Epidemiology. 2013;37:998–1002.
22. Cao MQ, You AB, Zhu XD, Zhang W, Zhang YY, Zhang SZ, et al. miR-182-5p promotes hepatocellular carcinoma progression by repressing FOXO3a. J Hematol Oncol. 2018;11:12.
23. Du C, Weng X, Hu W, Lv Z, Xiao H, Ding C, et al. Hypoxia-inducible MiR-182 promotes angiogenesis by targeting RASA1 in hepatocellular carcinoma. J Exp Clin Cancer Res. 2015;34:67.
24. Qin J, Luo M, Qian H, Chen W. Upregulated miR-182 increases drug resistance in cisplatin-treated HCC cell by regulating TP53INP1. Gene. 2014; 538:342–7.
25. Wang Q, Yu X, Li Q, Qin L, Tan S, Zeng X, et al. Association between miR-199a rs74723057 and MET rs1621 polymorphisms and the risk of hepatocellular carcinoma. Oncotarget. 2016;7:79365–71.
26. An J, Liu J, Liu L, Liu Y, Pan Y, Huang M, et al. A genetic variant in primary miR-378 is associated with risk and prognosis of hepatocellular carcinoma in a Chinese population. PLoS One. 2014;9:e93707.
27. Chu YH, Hsieh MJ, Chiou HL, Liou YS, Yang CC, Yang SF, et al. MicroRNA gene polymorphisms and environmental factors increase patient susceptibility to hepatocellular carcinoma. PLoS One. 2014;9:e89930.
28. Xu T, Zhu Y, Wei QK, Yuan Y, Zhou F, Ge YY, et al. A functional polymorphism in the miR-146a gene is associated with the risk for hepatocellular carcinoma. Carcinogenesis. 2008;29:2126–31.
29. Liu Y, Zhang Y, Wen J, Liu L, Zhai X, Liu J, et al. A genetic variant in the promoter region of miR-106b-25 cluster and risk of HBV infection and hepatocellular carcinoma. PLoS One. 2012;7:e32230.
30. Bei C, Liu S, Yu X, Qiu M, Tang B, Liao W, et al. Single Nucleotide Polymorphisms in miR-122 Are Associated with the Risk of Hepatocellular Carcinoma in a Southern Chinese Population. Biomed Res Int. 2018;2018: 1540201.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.