RESEARCH ARTICLE

The miR-146a rs2910164 G > C Polymorphism and Susceptibility to Digestive Cancer in Chinese

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Abstract

Background: Several studies have reported the role of the miR-146a rs2910164 G > C polymorphism as a susceptibility factor for several digestive cancers. However, the results have been controversial. Therefore, we conducted the present meta-analysis to obtain the most reliable estimate of the association. Methods: PubMed, Embase and Web of Science databases were searched. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were extracted and pooled to assess the strength of the association between miR-146a rs2910164 G > C polymorphism and digestive cancer risk. A total of four eligible studies including 3,447 cases and 5,041 controls based on the search criteria were included. Results: We observed that miR-146a rs2910164 G > C polymorphism was not significantly correlated with digestive cancer risks when all studies were pooled into the meta-analysis. While we found that miR-146a rs2910164 polymorphism was not associated with gastric cancer, it was significantly linked with hepatocellular cancer risk (the homozygote codominant model: OR = 1.40, 95% CI = 1.04-1.87). In the stratified analysis by ethnicity, significant associations were observed in Chinese population for the allele contrast model (OR = 1.25; 95% CI = 1.12-1.38), for the homozygote codominant model (OR = 1.62; 95% CI = 1.28-2.04), and for the recessive model (OR = 1.38; 95% CI = 1.16-1.64). However, studies with Asian groups presented no significant association for all genetic models. Conclusions: This meta-analysis suggests that the miR-146a rs2910164 G > C polymorphism is a low-penetrant risk factor for digestive cancers in Chinese.

Keywords: MicroRNAs - genetic polymorphisms - mutation - risk - meta-analysis - Chinese

Asian Pacific J Cancer Prev, **14 (1)**, 399-403

Introduction

It is well known that we divided RNA into protein-coding and non-coding RNA. Over the past decade, the field of RNA research has rapidly expanded. Many studies found that mRNA accounted for only 2% of all transcripts, and the remaining 98% are non-coding RNAs in the human genome (Lander et al., 2001; Esteller et al., 2011), suggesting that the noncoding portion of the genome is of crucial importance in the development of normal tissue development and disease (Wright et al., 2011). One of the members of these RNAs, microRNAs, an endogenous, small non-coding and 18 – 25 nucleotides RNAs, have a focus on this stage researches. They are encoded in the genome and are generally transcribed by RNA polymerase II, and exert their effects by associating with a group of proteins termed the 'RNA-induced silencing complex' (RISC). RISC is directed to target mRNAs via imperfect sequence complementarity between the miRNA and 3'-untranslated region (3'-UTR) of target mRNAs. In almost all studied examples, the targeting of a transcript by RISC leads to down-regulated gene expression through mRNA cleavage or translation inhibition (Gregory et al., 2004). MiRNAs have been shown to play crucial roles in diverse biological processes, such as cell apoptosis, differentiation, development, signal transduction (Bartel et al., 2004; Denli et al., 2004). Watson–Crick complementarity between the target and the seed region (2–8 nucleotides) of the mature miRNA is both necessary and sufficient for targeting and regulating of mRNAs by miRNAs. But the seed region of miRNAs is so short, so its polymorphism may affect the combination of the core area of binding the 3'UTR of target genes, thus affecting its regulation of target genes (Hu et al., 2008).

A *Homo sapiens* miR-146a gene located on chromosome, it has been reported that it play a vital role in several human cancers. Recently, single nucleotide polymorphism (SNP, rs2910164) has been identified in the miR-146a gene. More recently, Several studies have assessed the relationship between the polymorphism of miR-146a G > C and the risks to digestive cancers, however, the results have been controversial (Xu et al., 2008; Guo et al., 2010; Okubo et al., 2010; Srivastava et al., 2010; Zeng et al., 2010; Akkız et al., 2011; Hishida et al., 2011; Min et al., 2011; Zhang et al., 2011; Zhou et al., 2011). To derive a more precise effect on the association between miR-146a polymorphism and digestive cancers risks. Therefore, we conducted this meta-analysis.

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Materials and Methods

Identification and eligibility of relevant studies

Search terms "microRNA-146a", "digestive cancer", "genotype", "polymorphism" and "variant" were employed to explore publications in PubMed, ISI Web of Knowledge and Embase databases for relevant reports (last search update February 2012). We did not define any minimum number of patients to be included for meta-analysis. When multiple studies of the same patient population were identified, we included the published report with the largest sample size.

Inclusion and exclusion criteria

The following inclusion criteria were used to select literatures for this analysis: (a) evaluation of miR-146a rs2910164 G > C polymorphism and several digestive cancers, (b) only the case-control studies were considered, (c) sufficient published data for estimating an odds ratio (OR) with 95% confidence interval (CI). Major exclusion criteria were: (1) no control population, (2) no available genotype frequency, and (3) duplicated studies.

Data extraction

For each study, the following data were collected: first author's surname, year of publication, country of origin, ethnicity, criteria of enrolled patients, genotyping method, total numbers of cases and controls as well as numbers of cases and controls with GG, GC and CC genotypes. The strength of the association between miR-146a rs2910164 G > C polymorphism and digestive cancers risks were estimated using OR, with the corresponding 95% CI. Disagreement was resolved by discussion until a consensus was reached between the two authors. We did not define any minimum number of patients for inclusion in our meta-analysis.

Statistical methods

The risk of UC associated with miR-146a rs2910164 G > C was estimated for each study by OR with 95% CI. For five studies, we analysed the relationship for the allele contrast model (G vs C). At the same time, due to lack the specific genotypes of Glas's literature, so we estimated the association under other four different types of ORs, namely the homozygote codominant model (GG vs CC), the heterozygote codominant model (GC vs CC), the dominant model (GG+GC vs CC) and the recessive model (GG vs GC+CC). Hardy–Weinberg equilibrium (HWE) was tested by the Chi-square test. The Q-statistic was used to investigate the degree of heterogeneity between the trials, and a P-value 0.10 for the Q-test indicated a lack of heterogeneity among studies. Genotype distribution of controls in all studies was consistent with HWE, except for Zhang XW's study on HCC. We used the fixed-effects model and the random-effects model based on the Mantel–Haenszel method (Jose et al., 2008) and the DerSimonian and Laird method (Kjellsson et al., 2008), respectively, to combine values from each of the studies. A sensitivity analysis was also performed by omitting each study in turn to identify potential outliers. All statistical analyses were performed with Review Manage version 4.3 and STATA version 12.0 using two-sided P-values.

Results

Study characteristics

We obtained 10 studies about the association between miR-146a rs2910164 polymorphism. Following the above inclusion and exclusion criteria, 9 publications were included in the final meta-analysis (Xu et al., 2008; Guo et al., 2010; Okubo et al., 2010; Srivastava et al., 2010; Zeng et al., 2010; Akkız et al., 2011; Hishida et al., 2011; Min et al., 2011; Zhang et al., 2011; Zhou et al., 2011). Characteristics of studies focusing on miR-146a rs2910164 G > C are summarized in Table 1. Genotypes and separate P values for miR-146a rs2910164 polymorphism are list in Table 2. As shown in Figure 1, the literatures do not exist in significant heterogeneity.

Table 1. Characteristics of Studies Included in the Meta-analysis

First author/ Published year	Ethnicity	Method	Cancer type	Cases	Controls	HWE of controls
Xu, 2008	Chinese	PCR-RFLP	HCC	479	504	0.12
Akkiz, 2011	Turkish	PCR-RFLP	HCC	222	222	0.38
Zhang, 2011	Chinese	PIRA-PCR	HCC	925	1593	0.02
Zhou, 2011	Chinese	PCR-RFLP	HCC	186	483	0.06
Hishida, 2010	Japanese	PCR-CTPP	GC	584	1637	0.74
Okubo, 2010	Japanese	PCR-RFLP	GC	552	697	0.28
Zeng, 2010	Chinese	PCR-RFLP	GC	304	304	0.12
Guo, 2010	Chinese	PCR- Snapshot	ESCC	444	468	0.12
Min, 2011	Korean	PCR-RFLP	CRC	446	502	0.06
Srivastava, 2010	Indian	PCR-RFLP	GBC	230	224	0.08

HCC, hepatocellular cancer; GC, gastric cancer; ESCC, esophageal squamous cell carcinoma; CRC, colorectal cancer; GBC, gallbladder cancer

Table 2. Genotypes and P-values of for miR-146a rs2910164 Polymorphisms Included in the Meta-analysis

First	Cases					Controls				
author	GG	GC	CC	G	C	GG	GC	CC	G	C
Xu	80	241	158	401	557	58	249	197	365	643
Akkiz	137	75	10	349	95	144	67	11	355	89
Zhang	156	450	319	762	1088	296	725	577	1307	1879
Zhou	33	86	67	152	220	71	254	158	396	570
Hishida	82	272	230	436	732	229	775	633	1233	2041
Okubo	73	243	236	389	715	121	322	254	564	830
Zeng	62	153	89	277	331	53	132	119	238	370
Guo	234	190	20	658	230	206	220	42	632	304
Min	62	233	151	357	535	69	245	188	383	621
Srivastava	129	90	11	348	112	138	81	5	357	91

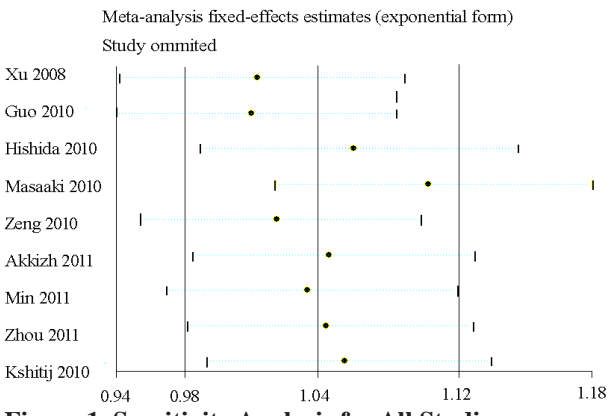

Figure 1. Sensitivity Analysis for All Studies

Table 3. Results of Meta-analysis for miR-146a rs2910164 Polymorphisms and Digestive System Cancer Risks

Study groups	Variables[a]	Allele contrast model		Homozygote codominant	
		OR (95% CI)	P_h	OR (95% CI)	P_h
Total	9	1.05 (0.92-1.20)	0	1.15 (0.86-1.53)	0.001
Two major digestive cancer types					
HCC	3	1.08 (0.89-1.32)	0.128	1.40 (1.04-1.87)[a]	0.308
GC	3	1.00 (0.78-1.27)	0.003	0.98 (0.63-1.53)	0.009
Ethnicity					
Asian	8	1.06 (0.92-1.22)	0	1.15 (0.85-1.57)	0
Chinese	4	1.25 (1.12-1.38)[a]	0.222	1.62 (1.28-2.04)[a]	0.237

Study groups	Heterozygote codominant		Dominant model		Recessive model	
	OR (95% CI)	P_h	OR (95% CI)	P_h	OR (95% CI)	P_h
Total	1.08 (0.90-1.30)	0.018	1.10 (0.89-1.34)	0.001	1.06 (0.89-1.27)	0.016
Two major digestive cancer types						
HCC	1.06 (0.85-1.31)	0.208	1.13 (0.92-1.38)	0.186	1.19 (0.85-1.68)	0.108
GC	1.04 (0.76-1.42)	0.014	1.03 (0.73-1.44)	0.003	0.94 (0.72-1.25)	0.114
Ethnicity						
Asian	1.07 (0.89-1.30)	0.01	1.10 (0.88-1.36)	0.001	1.08 (0.90-1.31)	0.014
Chinese	1.25 (0.91-1.71)	0.037	1.34 (0.98-1.82)	0.029	1.38 (1.16-1.64)[a]	0.811

P_h, *P*-value of overall effect test; [a]Statistically significant results

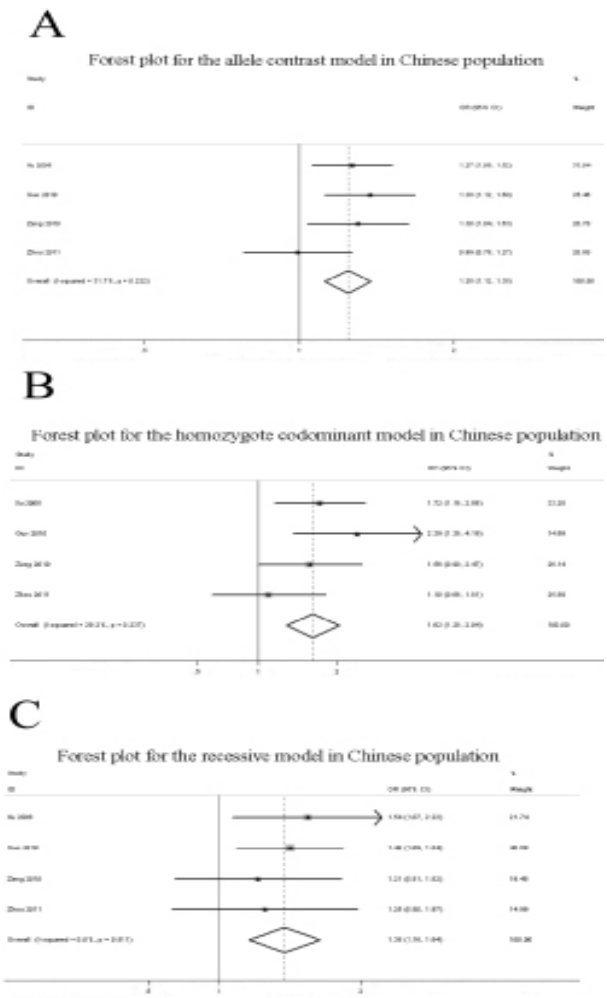

Figure 2. Forest Plot for the Association Between miR-146a rs2910164 G > C and Digestive Cancers Risks in Chinese Population Studies. A. for the allele contrast model; B. for the homozygote codominant model; C. for the recessive model). The study is shown by the point estimate of the OR (the size of the square is proportional to the weight of each study) and 95% CI for OR; pooled OR and 95% CI have been appropriately derived from the fixed effects model

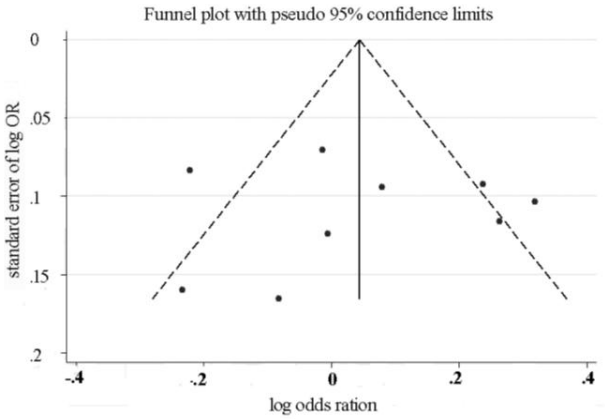

Figure 3. Funnel Plot Analysis for Odds Ratios of G Allele Compared with C Allele in Overall

Meta-analysis results

Table 3 lists the main results of pooled ORs for miR-146a rs2910164 G > C polymorphism and several digestive cancers risks. The results showed that no statistically significant difference were found in the allele and other genotype models of miR-146a rs2910164 G > C polymorphism among digestive cancers including gastric cancer and controls when all studies were pooled into the meta-analysis. Tumor location was taken into consideration for subgroup analysis, we observed that miR-146a rs2910164 polymorphism was not associated with gastric cancer, but it was significantly correlated with hepatocellular cancer risk (the homozygote codominant model: OR = 1.40, 95% CI = 1.04 – 1.87, *P* = 0.308 for heterogeneity, Figure 1). In the stratified analysis by ethnicity, significant associations were observed in Chinese population for the allele contrast model (OR = 1.25; 95% CI = 1.12 – 1.38; *P* = 0.222 for heterogeneity, Figure 2 A), for the homozygote codominant model (OR = 1.62; 95% CI = 1.28 – 2.04; *P* = 0.237 for heterogeneity, Figure 2 B), and for the recessive model (OR = 1.38; 95% CI = 1.16 – 1.64; *P* = 0.811 for heterogeneity, Figure

2 C). However, studies with Asian group presented no significant association for all genetic models.

When we conducted the above analysis, Zhang's study on HCC was excluded due to its HWE disequilibrium ($P = 0.02$) in controls.

Publication bias

We performed Begg's funnel plot and Egger's test to assess the publication bias of literatures. The results did not show any evidence of publication bias in all the comparisons. We present funnel plot for ORs of C versus G in Figure 3. Also, the results of Egger's test still did not suggest any evidence of publication bias ($P = 0.821$ for C vs G; $P = 0.796$ for GC vs CC; $P = 0.597$ for GG vs. CC, respectively).

Discussion

MiRNAs are probable regulators of varieties of physiological and pathological processes. Mutation and abnormal expression can effect on carcinogenesis and tumor progression. Some SNPs in pre-microRNAs, flanking regions or target sites have been demonstrated to affect certain physiological processes or related with diseases (Gong et al., 2012). Sometimes, single point mutations in the 7mer seed sites of miRNAs may reduce effectiveness or abolish miR mediated repression may induce effectiveness or abolish miR mediated repression (Richardson et al., 2011).

Some researchers have examined the association of miR-146a rs2910164 G > C polymorphism with several cancers risks, and a significant relationship was observed in several but not all studies (Xu et al., 2008; Guo et al., 2010; Okubo et al., 2010; Srivastava et al., 2010; Zeng et al., 2010; Akkız et al., 2011; Hishida et al., 2011; Min et al., 2011; Zhang et al., 2011; Zhou et al., 2011). Such as, some studies have divided cases and controls into HBV infection according to HBV DNA amount, and some researchers have taken into account age and other factors, so each obtained the different results.

The aim of our study was to demonstrate the role of miR-146a rs2910164 G > C polymorphism in the relationship with digestive system cancers risks using the meta-analysis. Interestingly, we observed that was not associated with gastric cancer, but it was significantly correlated with hepatocellular cancer risk. Simultaneously there was an evidence to indicate that miR-146a rs2910164 G > C polymorphism was associated with increased risk of digestive cancers in Chinese population. Of course, we must be noted that our study population was small, so it is still necessary to conduct larger sample studies considering gene-gene and gene-environment interactions, and using standardized unbiased genotyping methods, homogeneous gastric cancer patients, and sufficiently matched controls.

Recently, Genome-wide association studies (GWAS) help scientists understand the inheritance patterns of disorders on a global scale. It has identified scores of genetic variants that appear to contribute to human disease risk. Especially, it can led to an enormous boost in the identification of susceptibility genes for several diseases, but there are some insurmountable problems, the most critical is that some high-risk sites for low-frequency masked by low-risk sites for high-frequency, so this may result in the analysis is not comprehensive, and lose a lot of meaningful SNP sites.

In summary, our study shows a genetic association between miR-146a rs2910164 G > C variant increases susceptibility to digestive cancers risk in Chinese population, whereas we need to be further evaluated in larger sample collections.

Acknowledgements

The author(s) declare that they have no competing interests. This research was supported in part by grants from Project supported by the National Natural Science Foundation of China (Grant No. 81101579).

References

Akkız H, Bayram S, Bekar A, et al (2011). No association of pre-microRNA-146a rs2910164 polymorphism and risk of hepatocellular carcinoma development in Turkish population: a case-control study. *Gene*, **486**, 104-9.

Bartel DP (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, **116**, 281-97.

Denli AM, Tops BJ, Plasterk PH, Ketting RF and Hannon GJ (2004). Processing of primary microRNAs by the microprocessor complex. *Nature*, **432**, 231-5.

Esteller M (2011). Non-coding RNAs in human disease. *Nat Rev Genet*, **12**, 861-74.

Gong J, Tong Y, Zhang HM, et al (2012). Genome-wide identification of SNPs in microRNA genes and the SNP effects on microRNA target binding and biogenesis. *Hum Mutat*, **33**, 254-63.

Gregory RI, Yan K, Amuthan G, et al (2004). The microprocessor complex mediates the genesis of microRNAs. *Nature*, **432**, 235-40.

Guo H, Wang K, Xiong G, et al (2010). A functional varient in microRNA-146a is associated with risk of esophageal squamous cell carcinoma in Chinese Han. *Fam Cancer*, **9**, 599-603.

Hishida A, Matsuo K, Goto Y, et al (2011). Combined effect of miR-146a rs2910164 G/C polymorphism and Toll-like receptor 4 +3725 G/C polymorphism on the risk of severe gastric atrophy in Japanese. *Dig Dis Sci*, **56**, 1131-7.

Hu Z, Chen J, Tian T, et al (2008). Genetic variants of miRNA sequences and non-small cell lung cancer survival. *J Clin Invest*, **118**, 2600-8.

Jose S, George PS, Mathew A (2008). Assessment of confounding and interaction using the mantel-haenszel risk estimation method. *J Cancer Prev*, **9**, 323-5.

Kjellsson MC, Zingmark PH, Jonsson EN, Karlsson MO (2008). Comparison of proportional and differential odds models for mixed-effects analysis of categorical data. *J Pharmacokinet Pharmacodyn*, **35**, 483-501.

Lander ES, Linton LM, Birren B, et al (2001). Initial sequencing and analysis of the human genome. *Nature*, **409**, 860-921.

Min KT, Kim JW, Jeon YJ, et al (2011). Association of the miR-146aC>G, 149C>T, 196a2C>T, and 499A>G polymorphisms with colorectal cancer in the Korean population. *Mol Carcinog*, **51**, E65-73.

Okubo M, Tahara T, Shibata T, et al (2010). Association between common genetic variants in pre-microRNAs and gastric cancer risk in Japanese population. *Helicobacter*, **15**, 524-31.

Richardson K, Lai CQ, Parnell LD, Lee YC, Ordovas JM (2011). A genome-wide survey for SNPs altering microRNA seed sites identifies functional candidates in GWAS. *BMC Genomics*, **12**, 504.

Srivastava K, Srivastava A, Mittal B (2010). Common genetic variants in pre-microRNAs and risk of gallbladder cancer in North Indian population. *J Hum Genet*, **55**, 495-9.

Wright MW, Bruford EA (2011). Naming 'junk': human non-protein coding RNA (ncRNA) gene nomenclature. *Hum Genomics*, **5**, 90-8.

Xu T, Zhu Y, Wei QK, et al (2008). A functional polymorphism in the miR-146a gene is associated with the risk for hepatocellular carcinoma. *Carcinogenesis*, **29**, 2126-31.

Zeng Y, Sun QM, Liu NN, et al (2010). Correlation between pre-miR-146a C/G polymorphism and gastric cancer risk in Chinese population. *World J Gastroenterol*, **16**, 3578-83.

Zhang XW, Pan SD, Feng YL, et al (2011). Relationship between genetic polymorphism in microRNAs precursor and genetic predisposition of hepatocellular carcinoma. *Zhonghua Yu Fang Yi Xue Za Zhi*, **45**, 239-43.

Zhou J, Lv R, Song X, et al (2011). Association Between Two Genetic Variants in miRNA and Primary Liver Cancer Risk in the Chinese Population. *DNA Cell Biol*, **31**, 524-30.