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Original Article

Functional polymorphisms in two pre-microRNAs and cancer risk: a meta-analysis

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Abstract: Since the identification of two functional polymorphisms in pre-miRNAs (*miR-146a* rs2910164 and *miR-196a2* rs11614913), a number of studies were published in the past several years to evaluate the associations between the two SNPs and cancer risk. However, the findings remain conflicting rather than conclusive. This meta-analysis included 12 published case-control studies, 5,916 cancer patients and 6,869 control subjects for SNP rs2910164, and 6,574 cases and 7,601 controls for SNP rs11614913. The allele C of rs11614913 was associated with a significantly increased risk of overall cancers (CC vs. TT: OR=1.18; 95%CI: 1.02-1.37; CT vs. TT: OR=1.11; 95% CI: 1.02-1.21; CC/CT vs. TT: OR=1.13; 95%CI: 1.04-1.23). However, we failed to find main effects for rs2910164 on overall cancer risk in different genetic models tested, while significantly increased risk was evident among cancers other than breast (GG/GC vs. CC: OR=1.30, 95%CI: 1.03-1.64; between study heterogeneity test: P = 0.007). The two functional SNPs may represent tissue specific effect on cancer susceptibility; however, additional well-designed large studies are required for the validation of the associations.

Keywords: miRNA, polymorphism, cancer, susceptibility , meta-analysis

Introduction

Lots of efforts have been made in the field of cancer research and enormous genes were found to be involved in carcinogenesis. Recently, the addition of noncoding RNAs to cancer etiopathogenesis has provided stimulus for further researches. MicroRNAs (miRNAs) are about 20-nucleotide-long small noncoding RNAs, which control gene activity and affect the expression of proteins by base pairing with target mRNAs at the 3'-untranslated regions (3'UTR), leading to mRNA cleavage or translational repression [1-3]. It has been proposed that miRNAs are involved in various biological processes [4], and the information obtained to date has suggested that miRNA may play a vital important role in carcinogenesis [5-9]. Genes encoding miRNAs located in chromosomal regions that are amplified in cancers can function as oncogenes, while those in regions deleted in cancers may act as tumor suppressors [9-11]. Newly published evidence has also shown that a global reduction in miRNA processing was cancer prone, and miRNA profiling have been successfully applied to classify tumors [10-13].

It has been reported that SNPs (single nucleotide polymorphism) or mutations located in miRNA regions might change the processing of the miRNA as well as alter the target binding affinity and specificity [14]. In 2008, Jazdzewski *et al*. found that the SNP rs2910164 in *pre-miR-146a* was associated with mature *miR-146a* expression and differently influenced target report gene levels [15]. Likewise, Xu *et al*. showed rs2910164GG conferred a higher expression level of mature *miR-146a* by *in vitro* cell model compared with the CC genotype [16]. However, in the contrary, Shen *et al*. suggested that breast/ovarian cancer patients carrying the C allele of rs2910164 had high levels of mature

miR-146 than the G allele [17]. For rs11614913 in *miR-196a2*, in a genotype-phenotype correlation analysis of lung cancer tissues, homozygote rs11614913CC was associated with a significant increased mature *miR-196a* expression but without changes in the levels of its precursor [18]. The same study also revealed that rs11614913 could affect the binding of mature *miR-196a2-3p* to its target mRNA [18]. Furthermore, Hoffman *et al.* reported similar evidence that the risky *miR-196a2*-C allele led to more efficient processing of the miRNA precursor to its mature form as well as enhanced capacity to regulate target genes [19].

For the past several years, emerging molecular epidemiological studies studied the associations of the two functional SNPs (*miR-146a* rs2910164 and *miR-196a2* rs11614913) and susceptibility of diverse cancers in different populations. The tumor types in the case populations included lung, breast, prostate, bladder, renal, gastric, glioma, thyroid, hepatocellular and esophageal [15,16,19-29]. However, these published studies presented contradicting findings. Considering the amount of accumulated published data are available, it is necessary for us to carry out a systematic review and meta-analysis to assess the overall tumor risk associated with SNPs rs2910164 and rs11614913 and to quantify the potential between-study heterogeneity.

Materials and methods

Identification and eligibility of relevant studies

We have attempted to include all the case-control studies published to date on cancers with genotyping data for at least one of the two SNPs (*miR-146a* rs2910164 and *miR-196a2* rs11614913). In order to obtain all possible articles we need, we searched the electronic literature PubMed for relevant reports (last search update April 20th, 2010, using the search terms "miRNA or microRNA and cancer and polymorphism") by two independent investigators (T.T. and Y.X.) and also did hand search from the references of related articles. Overall, we obtained thirteen published articles focused on the relationship between the two SNPs and tumor risk. However, one study was excluded because of the lack of detailed genotyping information [23]. Finally, the data for this analysis were available from twelve case-control studies, including 5,916 cancer cases and 6,869 controls for *miR-146a* rs2910164 (from 8 studies), and 6,574 cancer cases and 7,601 controls for *miR-196a2* rs11614913 (from 9 studies).

Data Extraction

Two investigators independently extracted data and reached consensus on all of the items. Data collected from these articles included the first author's name, year of publication, country of origin,ethnicity, type of cancer, number of cases and controls, genotype frequencies for cases and controls, characteristics of cancer cases and controls, and racial descent.

Statistical-analysis

We used the fixed-effect model and the random-effect model based on the Mantel-Haenszel method and the DerSimonian-Laird method, respectively, to combine values from single study [30]. When the effects were assumed to be homogenous, the fixed -effects model was used; otherwise, the random-effects model was more appropriate. Statistical heterogeneity was assessed with the $\chi 2$-based Q test, and the heterogeneity was considered significant when $P < 0.1$ [31]. Subgroup analyses were processed, according to tumor type [categorized as breast cancer and other cancers (only breast cancer has more than two published studies)] and ethnicity (categorized as Asian and Caucasian descents). The inter-study variance ($\tau 2$) was used to quantify the degree of heterogeneity between studies and the percentage of $\tau 2$ was used to describe the extent of explained heterogeneity [31]. Egger's test was utilized to provide diagnosis of publication bias (Linear regression analysis [32]). All analyses were done by using the Statistical Analysis System software (v.9.1.3; SAS Institute, Cary, NC, USA) and STATA7.0 (Stata-Corp, College Station, TX, USA). All the P values were two-sided.

Results

Characteristics of the studies

We established a database according to the extracted information from each study (**Table 1**). There are totally 8 case-control studies concerning SNP rs2910164, and 9 for SNP rs11614913. All studies indicated that the distribution of genotypes in the controls was con-

Table 1. Characteristics of literatures included in the meta-analysis

Author	Year	Origin	Ethnicity	Tumor type	Sample size (case/control)	HWE	MAF in controls	Power [b]
Horikawa	2008	American	Caucasian	Renal cell cancer	276/277(261/235)	0.024(0.648)	0.576(0.736)	0.182(0.139)
Jazdzewski	2008a[a]	Finland	Caucasian	Papillary thyroid cancer	(206/274)	(0.915)	(0.739)	(0.133)
Jazdzewski	2008b[a]	Poland	Caucasian	Papillary thyroid cancer	(201/475)	(0.661)	(0.774)	(0.141)
Jazdzewski	2008c[a]	American	Caucasian	Papillary thyroid cancer	(201/152)	(0.508)	(0.763)	(0.104)
Xu	2008	Chinese	Asian	Hepatocellular cancer	(479/504)	(0.119)	(0.362)	(0.284)
Yang	2008	American	Caucasian	Bladder cancer	736/731(691/674)	0.329(0.137)	0.586(0.763)	0.399(0.287)
Hoffman	2009	American	Caucasian	Breast cancer	426/466	0.583	0.602	0.260
Hu	2009	Chinese	Asian	Breast cancer	1009/1093(1009/1093)	0.207(0.221)	0.436(0.417)	0.548(0.545)
Peng	2009	Chinese	Asian	Gastric cancer	213/213	0.936	0.514	0.153
Tian	2009	Chinese	Asian	Lung cancer	1058/1035(1058/1035)	0.700(0.853)	0.453(0.406)	0.549(0.541)
Catucci	2010a[a]	German	Caucasian	Breast cancer	1101/1496(805/904)	0.711(0.753)	0.623(0.769)	0.594(0.339)
Catucci	2010b[a]	Italian	Caucasian	Breast cancer	751/1243(754/1243)	0.315(0.019)	0.649(0.732)	0.458(0.402)
Dou	2010	Chinese	Asian	Glioma	643/656	0.119	0.451	0.374
Qi	2010	Chinese	Asian	Hepatocellular cancer	361/391	0.869	0.487	0.238
Xu	2010	Chinese	Asian	Prostate cancer	(251/280)	(0.191)	(0.461)	(0.181)

[a] Different populations from one study.

[b] Power was calculated by the DSTPLAN4.2 software

NOTE: The data given in parenthesis were related to rs2910164, others were related to rs11614913.

Table 2. Associations of the two polymorphisms and cancer risk

		mir-146a rs2910164 OR(95%CI)							*mir-196a2* rs11614913 OR(95%CI)						
		No. of Comparisons	GG VS. CC	*P*	GC VS. CC	*P*	GG/GC VS. CC	*P*	No. of Comparisons	CC VS. TT	*P*	CT VS. TT	*P*	CC/CT VS. TT	*P*
Total	F	11	1.14(1.00-1.29)	0.048	1.07(0.97-1.19)	0.184	1.09(0.99-1.20)	0.082	10	1.17(1.06-1.29)	0.002	1.11(1.02-1.21)	0.018	1.13(1.04-1.23)	0.003
	R	11	1.19(0.96-1.48)	0.109	1.12(0.92-1.35)	0.256	1.12(0.94-1.33)	0.193	10	1.18(1.02-1.37)	0.027	1.11(1.02-1.22)	0.021	1.13(1.03-1.25)	0.010
Ethnicity															
Asian	F	4	1.22(1.04-1.43)	0.013	1.08(0.96-1.22)	0.174	1.12(1.00-1.25)	0.053	5	1.18(1.03-1.35)	0.018	1.10(0.98-1.23)	0.096	1.13(1.01-1.25)	0.027
	R	4	1.33(0.99-1.77)	0.056	1.08(0.97-1.22)	0.176	1.15(0.98-1.34)	0.088	5	1.16(0.94-1.43)	0.175	1.10(0.98-1.23)	0.096	1.13(1.01-1.25)	0.027
Caucasian	F	7	1.00(0.81-1.23)	0.966	1.03(0.83-1.28)	0.776	1.01(0.82-1.24)	0.934	5	1.15(1.00-1.33)	0.047	1.12(0.98-1.29)	0.087	1.14(1.00-1.30)	0.051
	R	7	1.08(0.78-1.50)	0.643	1.30(0.83-2.05)	0.256	1.17(0.80-1.70)	0.418	5	1.20(0.96-1.51)	0.102	1.16(0.96-1.40)	0.116	1.19(0.97-1.46)	0.095
Tumor type															
Breast	F	3	0.91(0.75-1.10)	0.311	0.95(0.81-1.11)	0.493	0.95(0.81-1.10)	0.471	4	1.25(1.09-1.43)	0.002	1.15(1.01-1.31)	0.033	1.19(1.05-1.34)	0.005
	R	3	0.91(0.75-1.09)	0.307	0.90(0.70-1.16)	0.424	0.92(0.75-1.12)	0.399	4	1.30(1.01-1.68)	0.041	1.17(0.98-1.40)	0.086	1.22(1.00-1.50)	0.053
Other [a]	F	8	1.36(1.15-.161)	0.000	1.17(1.03-1.34)	0.020	1.21(1.06-1.37)	0.004	6	1.09(0.95-1.26)	0.204	1.08(0.96-1.22)	0.206	1.09(0.97-1.21)	0.151
	R	8	1.44(1.10-1.89)	0.008	1.31(1.00-1.72)	0.047	1.30(1.03-1.64)	0.024	6	1.09(0.92-1.31)	0.324	1.08(0.96-1.22)	0.208	1.09(0.97-1.21)	0.152

F: Fixed-effects model, R: Random-effects model;
[a] Including cancers of renal, thyroid, bladder, lung, hepatocellular, and prostate for SNP *miR-146a* rs2910164; and cancers of glioma, bladder, renal, gastric, hepatocellular, and lung for SNP *miR-196a2* rs11614913

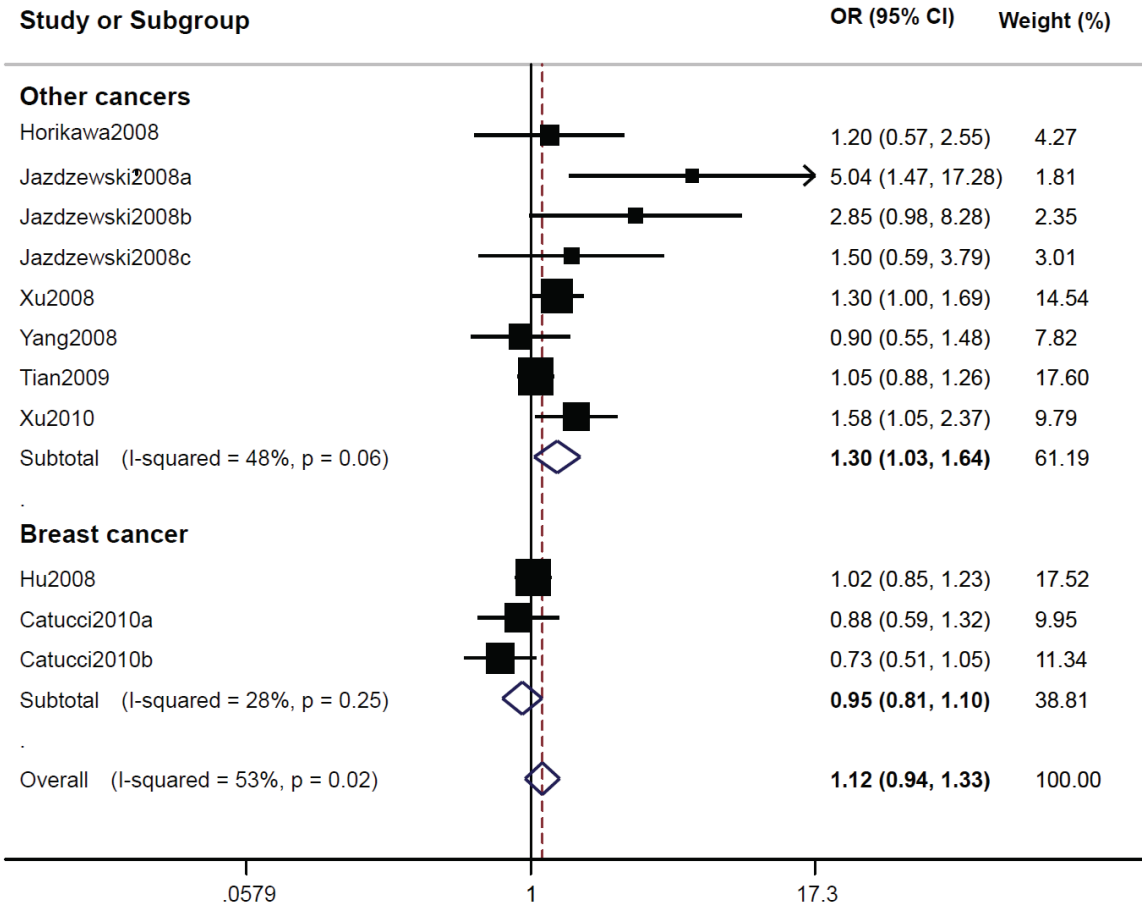

Figure 1. ORs (log scale) of different cancers associated with rs2910164 for the GG/GC genotypes compared with the CC genotype.

sistent with Hardy-Weinberg equilibrium, except for one study for rs2910164 [28] and one study for rs11614913 [26]. However, none of the comparisons had the statistical power greater than 80% when we assumed an allelic risk was 1.2 for the two SNPs.

Quantitative synthesis

MiR-146a rs2910164. There was a distinct variation in the allele frequency of rs2910164G across different ethnicities, ranging from 0.362 in an Asian population [16] to 0.774 in a Caucasian population [15]. The mean frequency of rs2910164G was 0.408 for Asians and 0.753 for Caucasians. In the meta-analysis, we estimated the risks of genotypes GG and GC, compared with CC, respectively, and then evaluated the risk of GG/GC versus CC. However, we failed to find significant main effects for rs2910164 on cancer risk in all genetic models tested (**Table 2**). In the stratified analyses, significantly increased risk was found among Asians by comparing GG/GC with CC (OR=1.12; 95% CI: 1.00-1.25; *P* = 0.15 for heterogeneity test) and in cancer patients other than breast cancer in all tested genetic models (GG vs. CC: OR=1.44; 95% CI: 1.10-1.89; *P* = 0.09 for heterogeneity test; GC vs. CC: OR=1.31; 95% CI: 1.00-1.72; *P* = 0.02 for heterogeneity test; and GG/GC vs. CC: OR=1.30; 95% CI: 1.03-1.64; *P* = 0.06 for heterogeneity test) (**Table 2**, **Figure 1**).

MiR-196a2 rs11614913. Similarly, there was a distinct variation in the allele frequency of rs11614913C across different ethnicities, ranging from 0.436 in an Asian population [20] to 0.649 in a Caucasian population [28]. The

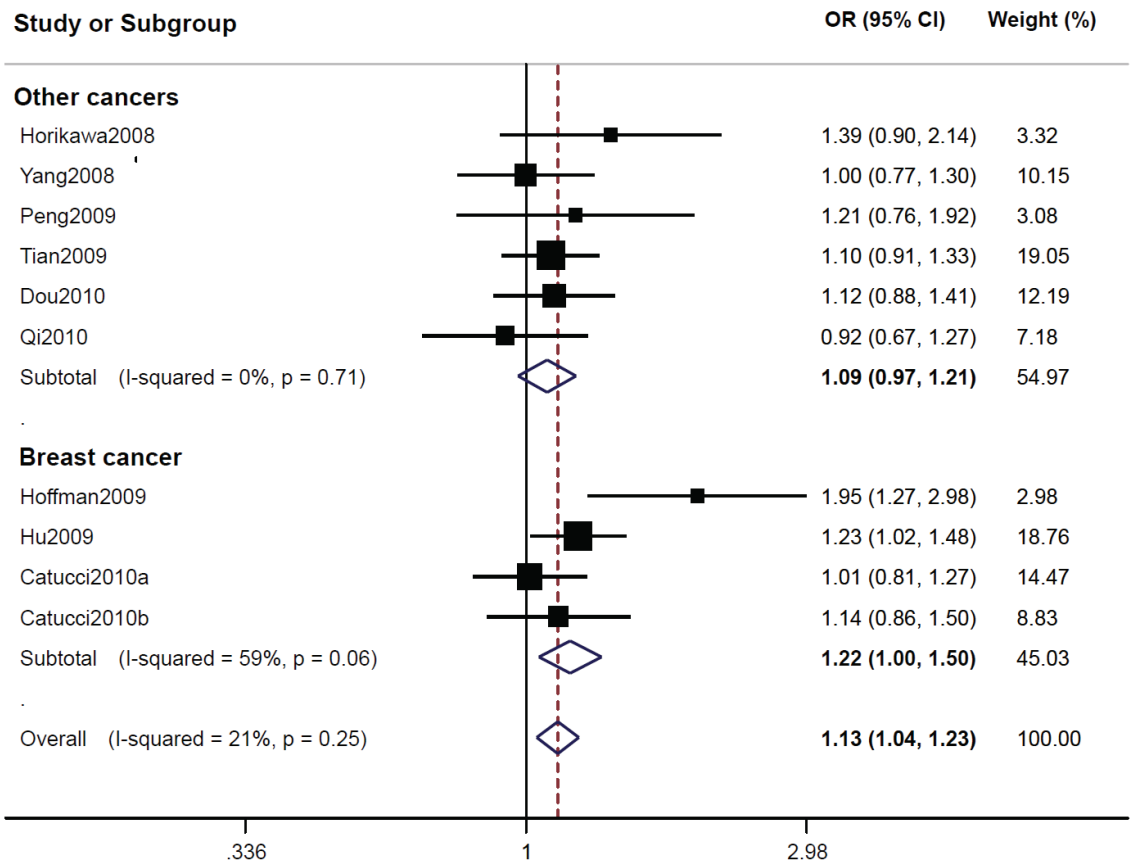

Figure 2. ORs (log scale) of different cancers associated with rs11614913 for the CC/CT genotypes compared with the TT genotype.

mean frequency of rs11614913C was 0.619 for Caucasians and 0.455 for Asians. When all the eligible studies were pooled, the genotypes including rs11614913C allele were associated with increased tumor risk in all genetic models tested (CC vs. TT: OR=1.18; 95% CI: 1.02-1.37; *P* = 0.03 for heterogeneity test; CT vs. TT: OR=1.11; 95% CI: 1.02-1.21; *P* = 0.41 for heterogeneity test; and CC/CT vs. TT: OR=1.13; 95% CI, 1.04-1.23; *P* = 0.25 for heterogeneity test; **Table 2**). In the subgroup analyses, we reached significantly elevated risks associated with the rs11614913C harboring genotypes for breast cancer in all models tested (CC vs. TT: OR=1.30; 95% CI: 1.01-1.68; *P* = 0.03 for heterogeneity test; CT vs. TT: OR=1.15; 95% CI: 1.01-1.31; *P* = 0.16 for heterogeneity test; and CC/CT vs. TT: OR=1.22; 95% CI: 1.00-1.50; *P* = 0.06 for heterogeneity test), while significantly increased risk was found among Asians by comparing CC/CT with TT (OR = 1.13; 95% CI, 1.01-1.25; *P* = 0.66 for heterogeneity test) (**Table 2**, **Figure 2**).

Test of between subgroup heterogeneity and source of between study heterogeneity

We also tested between subgroup heterogeneity and evaluated the source of between study heterogeneity for rs2910164 (GG versus CC) and rs11614913 (CC versus TT) by tumor type and ethnicity. We found that tumor type (χ^2 = 7.36, df = 1, *P* = 0.007) do contribute to substantial altered between subgroup heterogeneity, but not the ethnicity (χ^2 = 0.87, df = 1, *P* = 0.351) for SNP rs2910164. Furthermore, meta-regression analyses revealed that tumor type can explain 65.5% (*P* = 0.035) of the τ^2, whereas ethnicity cannot explain significant between study heterogeneity (*P* = 0.397). For rs11614913, neither tumor type (χ^2 = 1.41, df = 1, *P* = 0.235) nor ethnicity (χ^2 = 0.05, df = 1, *P*

= 0.830) contributed substantially to the between subgroup heterogeneity. Meta-regression analyses also revealed that none of these two factors could explain significant between study heterogeneity (tumor type: P = 0.342; ethnicity: P = 0.786). Similar results were not shown for other genetic models tested (GC VS.CC and GG/GC VS.CC for rs2910164 and CT VS.TT and CC/CT VS. TT for rs11614913).

Publication bias

We used Egger's test to access the publication bias of literatures. The result of Egger's test did not show any statistically significant evidence for publication bias for the two SNPs (rs2910164: t = 1.58, P = 0.15 for GG/GC vs. CC and rs11614913: t = 1.13, P = 0.29 for CC/CT vs. TT).

Discussion

Several studies have reported that the sequence variations in pre-miRNA may affect the maturation process of miRNAs and binding activity to their target mRNAs, including rs2910164 and rs11614913 [15-19, 29]. Jazdzewski *et al*. proposed the allele C of rs2910164 decreased *pri-miR-146a* nuclear processing efficiency, reduced production of mature miRNA and resulted in less efficient inhibition of the target genes including *TRAF6*, *IRAK1* and *PTC1* [15]. Other studies also showed the SNP rs2910164C allele was related to decreased production of mature *miR-146a* 16, 29, but Shen *et al.* got a contrary result for breast/ovarian cancer patients [17]. Interestingly, our meta-analysis found a significant association for rs2910164 and cancer risk other than breast and at least an apparently controversial risk estimation between the two subgroups (P for between subgroup heterogeneity: 0.007). Therefore, we think rs2910164 may affect tumor susceptibility in a tissue specific way with yet unidentified mechanism.

The allele C of rs11614913 also influenced endogenous processing of the miRNA precursors to its mature form and affected the binding of mature miRNA to its target genes [18, 19]. Furthermore, a significant association of SNP rs11614913 with G2 cell cycle delay has been found, which was functionally verified that the SNP play a important role in tumorigenesis [19]. In our meta-analysis, we found consistently increased breast cancer risk for the rs11614913C allele. However, the associations with other cancers warrant further studies. In stratified analyses by race, the associations between the two SNPs and cancer risk were significant in Asian but not in Caucasians and the between subgroup heterogeneity was not evident. Interestingly, the risk alleles of the two SNPs were rare in Asians, but common in Caucasians. Therefore, these two SNPs may truly causative SNPs instead of tagging ones.

An increasing number of studies have also reported that m*iR-146a* and *miR-196a* were associated with multiple kinds of malignant tumors. He *et al.* reported that *miR-146a* was up-regulated in patients with papillary thyroid carcinoma (PTC) [33]. Overexpression of *miR-146a* in PTC was also proved by one another study [15]. In addition, it has also been reported that *miR-146a* was highly expressed in pediatric acute leukemia [34]. Moreover, Pacifico *et al.* found that *miR-146a* was strongly up-regulated in human thyroid carcinoma FRO cells and the inhibition of *miR-146a* expression in FRO cells could decrease their oncogenic potential [35]. For *miR-196a*, which was up-regulated in breast cancer compared with normal breast [13], suggesting it might potentially act as oncogene in breast cancer. Highly elevated levels of *miR-196a* were also detected in other cancers, such as colorectal cancer [36] and esophageal adenocarcinoma [37]. Additionally, cell culture experiments supported high m*iR-196a* levels could supress the activities of various cancer related genes, such as *ANXA1* (annexin A1) [38], suppression of which was well documented in various cancers [39, 40].

In conclusion, considering the population evidence provided by this meta-analysis together with the functional evaluations on the two SNPs, we think it is clearer that the two SNPs (*miR-146a* rs2910164 and *miR-196a2* rs11614913) play a crucial role in cancer susceptibility. The functional relevant of the SNPs and their related miRNAs may represent tissue specific effect, especially *miR-146a* rs2910164.

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