The Association between Four Genetic Variants in MicroRNAs (rs11614913, rs2910164, rs3746444, rs2292832) and Cancer Risk: Evidence from Published Studies

Bangshun He1*, Yuqin Pan1*, William C. Cho2, Yeqiong Xu1, Ling Gu1, Zhenglin Nie1, Liping Chen1, Guoqi Song1, Tianyi Gao1, Rui Li1, Shukui Wang1*

1 Central Laboratory of Nanjing First Hospital, Nanjing Medical University, Nanjing, China, 2 Department of Clinical Oncology, Queen Elizabeth Hospital, Kowloon, Hong Kong

Abstract

MicroRNAs (miRNAs) participate in diverse biological pathways and may act as either tumor suppressor genes or oncogenes. Single nucleotide polymorphisms (SNPs) in miRNA may contribute to cancer development with changes in the microRNA's properties and/or maturation. Polymorphisms in miRNAs have been suggested in predisposition to cancer risk; however, accumulated studies have shown inconsistent conclusions. To further validate determine whether there is any potential association between the four common SNPs (miR-196a2C>T, rs11614913; miR-146aG>C, rs2910164; miR-499A>G, rs3746444; miR-149C>T, rs2292832) and the risk for developing risk, a meta-analysis was performed according to the 40 published case-control studies. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to assess the extent of the association. The results demonstrated that the rs11614913TT genotype was significantly associated with a decreased cancer risk, in particular with a decreased risk for colorectal cancer and lung cancer, or for Asian population subgroup. In addition, the rs2910164C allele was associated with decreased risk for esophageal cancer, cervical cancer, prostate cancer, and hepatocellular carcinoma (HCC), in particular in Asian population subgroup. Similarly, the rs3746444G allele was observed as a risk factor for cancers in the Asian population. It is concluded that two SNPs present in miRNAs(rs11614913TT, and rs2910164C) may protect against the pathogenesis of some cancers, and that the rs3746444 may increase risk for cancer.

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* E-mail: shukwang@163.com
† These authors contributed equally to this work.

Introduction

MicroRNAs (miRNAs) are small, single-stranded, 19-21 nucleotide long non-protein-coding RNA molecules, functioning as negative regulators that involve post-transcriptional gene expression through binding to their target mRNAs regions and consequently lead to mRNA cleavage or translational repression [1]. Accumulating evidence has shown that miRNAs regulate the expression of roughly 10–30% of the all human genes through post-transcriptional mechanisms [2], contributing to excessive physiologic and pathologic conditions, including cell differentiation, proliferation, and apoptosis [1], and in particular to the development and progression of various human cancers by regulating the expression of proto-oncogenes or tumor suppressor genes [3,4,5].

SNPs in miRNA genes are regarded to affect function by three ways: first, through the transcription of the primary transcript; second, through pri-miRNA and pre-miRNA processing; and third, through effects on miRNA-mRNA interactions [6]. Recently, several studies have demonstrated that some polymorphism(SNPs) present in the miRNA genes, which can alter miRNA expression and/or maturation and be associated with the development and progression of cancer [6]. For example, four SNPs—miR-196a2C>T (or rs11614913), miR-146aG>C (rs2910164), miR-499A>G (rs3746444), and miR-149C>T (rs2292832) – identified in the pre-miRNA regions of miR-146a, miR-149, miR-196a2, and miR-499, respectively, have been reported to be associated with cancer risk [7,8]. However, conclusions of the relevant studies remain inconsistent, in part because of heterogeneity of the cancer subtype, small sample size, and ethnicity of the patients. To further determine whether there is an association of the four SNPs in the miRNA genes with the risk for developing cancer, a comprehensive review and analysis of published data from different studies is needed. In this study, we have extensively reviewed literature and performed a meta-analysis based on all eligible case-control published data to
evaluate the association between the four polymorphisms and cancer susceptibility.

Materials and Methods

Identification of eligible studies

We carried out a search of the PubMed and Embase databases for all relevant reports using the key words ‘microRNA/miR-146a/miR-149/miR-196a2/miR-199’, ‘polymorphism’, and ‘cancer’ (updated to Jun 23, 2012). The search was limited to English language papers and human subject studies. We evaluated potentially relevant publications by examining their titles and abstracts, thereafter all studies matching the eligible inclusion criteria were retrieved. In addition, studies were identified by a manual search of the references listed in the reviews involved. All the studies were included if they met the following criteria: (i) about the rs11614913, rs2910164, rs3746444, and rs2292832 polymorphisms and cancer risk, (ii) from a case–control designed study, and (iii) genotype frequencies available.

Data extraction

All data complying with the selection criteria were extracted independently by two staff (B.S.H., and Y.Q.X). For each study, the following characteristics were extracted: the first author’s last name, year of publication, country of origin, ethnicity, the numbers of genotyped cases and controls, source of control groups (population- or hospital-based controls), genotyping methods and cancer type. Ethnic descents were categorized as Caucasian, Asian or mixed (which included more than one ethnic descent). One study included the information for genotype rs11614913 CT+TT, without the data for CT and TT genotypes, so we were only able to calculate the OR for the comparison between CT+TT vs. TT.

Statistical analysis

The four SNPs in miRNAs were tested for the associations with cancer susceptibility based on different genetic models. The meta-analysis examined the overall association of the four SNPs with the risk of cancer as measured by odds ratios (ORs) at the 95% confidence intervals (CIs). To contrast the wild-type homozygote (WW), we first estimated the risk of the rare allele homozygote (RR) and heterozygous (WR) genotypes on cancers, then evaluated the risk of cancer as measured by odds ratios (ORs) at the 95% confidence interval (CI). The heterogeneity between studies was evaluated by the Chi-square based Q statistical test [10], with heterogeneity (P) 0.90, P = 0.079). Subgroup analysis by the ethnicity revealed a significant association in the comparison of TT vs. CC (OR = 0.79, 95% CI: 0.65–0.96, P = 0.096). Subgroup analysis by ethnicity revealed a significant association between the polymorphism and cancer risk in both the hospital and population based controls for the comparison of TT vs. CC and TT vs. CT; moreover, a decreased risk was also observed for the comparison of TT vs. CC in hospital based study, as summarized in Table 2.

For the rs2910164 polymorphism, no significant risk association was observed in the overall pooled analysis. However, cancer type-subgroup analysis revealed a decreased risk for the comparison of GC vs. GG in the subgroup of HCC (OR = 0.95, 95% CI: 0.70–1.31), prostate cancer (OR = 0.77, 95% CI: 0.65–0.91, P = 0.425), cervical cancer (OR = 0.50, 95% CI: 0.37–0.68, P = 0.814) and esophageal cancer (OR = 0.38, 95% CI: 0.37–0.90, P = 0.053). Similarly, a decreased risk was observed for the comparison of GC vs. GG in the cervical cancer (OR = 0.71, 95% CI: 0.51–0.99, P = 0.254), CC+GC vs. GG in esophageal cancer (OR = 0.79, 95% CI: 0.65–0.96, P = 0.195), and GC vs. GG+GC in prostate cancer (OR = 0.63, 95% CI: 0.44–0.96, P = 0.699) and esophageal cancer (OR = 0.64, 95% CI: 0.41–0.98, P = 0.079). Subgroup analysis by ethnicity revealed a decreased
Table 1. Summary of published studies included.

| Author     | Year | Race   | Cancer type                | Control | Method                | Case/control Polymorphism site |
|------------|------|--------|----------------------------|---------|-----------------------|-------------------------------|
| Xu         | 2008 | Asian  | HCC                        | PB      | PCR-RFLP              | 479/504 rs2910164             |
| Hu         | 2008 | Asian  | Breast Cancer              | PB      | PCR-RFLP              | 1009/1093 rs11614913,rs2910164,rs3746444,rs2292832 |
| Jazdzewski | 2008 | Caucasian | Papillary thyroid carcinoma | PB      | SNPshot               | 608/901 rs2910164             |
| Ye         | 2008 | Caucasian | Esophageal Cancer          | PB      | SNPlex assay          | 307/388 rs11614913,rs2910164  |
| Horikawa   | 2008 | Caucasian | Renal cell carcinoma      | PB      | SNPlex assay          | 276/277 rs11614913,rs2910164  |
| Tian       | 2009 | Asian  | Lung Cancer                | PB      | PCR-RFLP              | 1058/1035 rs11614913,rs2910164,rs3746444,rs2292832 |
| Hoffman    | 2009 | mix    | Breast Cancer              | HB      | iPLEX GOLD           | 426/466 rs11614913            |
| Xu         | 2010 | Asian  | Prostate Cancer            | PB      | PCR-RFLP              | 251/280 rs2910164             |
| Yoo        | 2010 | Asian  | Lung cancer                | PB      | melting-curve analysis | 654/640 rs11614913           |
| Guo        | 2010 | Asian  | Esophageal cancer          | PB      | SNPshot               | 444/468 rs2910164             |
| Dou        | 2010 | Asian  | Glioma                     | PB      | LDR                   | 643/656 rs11614913            |
| Li         | 2010 | Asian  | HCC                        | HB      | PCR-RFLP              | 310/222 rs11614913            |
| Chen       | 2010 | Asian  | CRC                        | PB      | LDR                   | 126/407 rs11614913            |
| Pastrello  | 2010 | Caucasian | Breast/ovarian cancer     | PB      | PCR-RFLP              | 101/155 rs2910164             |
| Qi         | 2010 | Asian  | HCC                        | PB      | LDR                   | 361/391 rs11614913            |
| Peng       | 2010 | Asian  | Gastric Cancer             | PB      | PCR-RFLP              | 213/213 rs11614913            |
| Srivastava | 2010 | Asian  | Gallbladder cancer         | PB      | PCR-RFLP              | 230/230 rs11614913,rs2910164,rs3746444 |
| Zeng       | 2010 | Asian  | Gastric Cancer             | HB      | PCR-RFLP              | 304/304 rs2910164             |
| Catucci    | 2010 | Caucasian | Breast Cancer             | PB      | Taqman               | 1852/2739 rs11614913,rs2910164,rs3746444 |
| Liu        | 2010 | Caucasian | Head and neck cancer      | PB      | PCR-RFLP              | 1109/1130 rs11614913,rs2910164,rs3746444,rs2292832 |
| Christensen| 2010 | Caucasian | Head and neck cancer      | PB      | Taqman               | 484/555 rs11614913            |
| Okubo      | 2011 | Asian  | Gastric Cancer             | HB      | PCR-RFLP              | 552/697 rs11614913,rs2910164,rs3746444 |
| Zhou       | 2011 | Asian  | Cervical cancer            | PB      | PCR-RFLP              | 226/309 rs11614913,rs2910164,rs3746444 |
| Akkz       | 2011 | Caucasian | HCC                      | PB      | PCR-RFLP              | 185/185 rs11614913            |
| Zhu        | 2011 | Asian  | CRC                        | PB      | Taqman               | 573/588 rs11614913            |
| Permuth-Wey| 2011 | Caucasian | Glioma                    | PB      | Illumina's Golden Gate | 593/614 rs2910164         |
| Zhan       | 2011 | Asian  | CRC                        | HB      | PCR-RFLP              | 252/543 rs11614913            |
| Hong       | 2011 | Asian  | Lung Cancer                | PB      | Taqman               | 406/428 rs11614913            |
| Zhou       | 2011 | Asian  | Primary Liver Cancer       | PB      | PCR-RFLP              | 2910164,rs3746444            |
| Min        | 2011 | Asian  | CRC                        | PB      | PCR-RFLP              | 446/502 rs11614913,rs2910164,rs3746444,rs2292832 |
| Hishida    | 2011 | Asian  | Gastric Cancer             | HB      | PCR-CTPP              | 583/1637 rs2910164           |
| George     | 2011 | Asian  | Prostate cancer            | PB      | PCR-RFLP              | 159/230 rs11614913,rs2910164,rs3746444 |
| Mittal     | 2011 | Asian  | Bladder Cancer             | PB      | PCR-RFLP              | 212/250 rs11614913,rs2910164,rs3746444 |
| Akkz       | 2011 | Caucasian | HCC                      | PB      | PCR-RFLP              | 222/222 rs2910164            |
| Yue        | 2011 | Asian  | Cervical cancer            | PB      | PCR-RFLP              | 447/443 rs2910164             |
| Zhang      | 2011 | Asian  | Breast Cancer              | PB      | PCR-RFLP              | 248/243 rs11614913,rs2292832 |
| Jedlinski  | 2011 | Caucasian | Breast Cancer             | PB      | PCR-RFLP              | 187/171 rs11614913           |
| Zhou       | 2012 | Asian  | Gastric Cancer             | HB      | Taqman               | 1686/1895 rs2910164         |
| Xiang      | 2012 | Asian  | HCC                        | PB      | PCR-RFLP              | 100/90 rs2910164,rs3746444  |
| Kim        | 2012 | Asian  | HCC                        | PB      | PCR-RFLP              | 159/201 rs11614913,rs2910164,rs3746444,rs2292832 |

HB, hospital based; PB, population based; HCC, hepatocellular carcinoma; CRC, colorectal cancer; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism; PCR-CTPP, polymerase chain reaction with confronting two-pair primers; LDR, ligation detection reaction.

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Table 2. Stratification analyses of genetic susceptibility of rs11614913 polymorphism to cancer risk.

| Category              | Cases/ Controls | TT vs. CC | CT vs. CC | TT+CT vs. CC | TT vs. CC+CT |
|-----------------------|-----------------|-----------|-----------|--------------|--------------|
|                       |                 | OR(95% CI) | P         | OR(95% CI) | P       | OR(95% CI) | P       | OR(95% CI) | P     |
| Total                 | 12663/14739     | 0.83(0.74,0.93) | 0.001  | 0.98(0.90,1.07) | 0.004 | 0.94(0.86,1.03) | 0.001 | 0.86(0.79,0.95) | 0.005 |
| Cancer types          |                 |           |           |              |        |              |        |              |       |
| Breast cancer         | 3722/4712       | 0.81(0.61,1.09) | 0.014  | 0.94(0.85,1.04) | 0.532 | 0.91(0.83,1.00) | 0.148 | 0.87(0.70,1.08) | 0.027 |
| Colorectal cancer     | 1397/2040       | 0.70(0.57,0.85) | 0.284  | 0.81(0.65,1.08) | 0.367 | 0.77(0.65,0.91) | 0.377 | 0.80(0.69,0.94) | 0.198 |
| HCC                   | 1015/999        | 0.74(0.47,1.19) | 0.022  | 0.90(0.72,1.11) | 0.631 | 0.85(0.69,1.04) | 0.19  | 0.85(0.72,1.11) | 0.037 |
| Lung cancer           | 2118/2103       | 0.77(0.65,0.91) | 0.895  | 0.90(0.77,1.04) | 0.098 | 0.85(0.74,0.98) | 0.289 | 0.93(0.73,0.95) | 0.281 |
| Gastric cancer        | 765/910         | 0.80(0.61,1.06) | 0.306  | 0.84(0.65,1.08) | 0.163 | 0.82(0.65,1.04) | 0.162 | 0.89(0.72,1.11) | 0.069 |
| Other cancers         | 3646/3975       | 1.06(0.91,1.23) | 0.125  | 3.239         | 0.239 | 1.13(1.03,1.25) | 0.096 | 0.90(0.74,1.17) | 0.024 |
| Ethnicities           |                 |           |           |              |        |              |        |              |       |
| Asian                 | 7837/8878       | 0.80(0.73,0.88) | 0.169  | 23.7          | 0.001 | 57.4         | 0.001 | 58.3         | 0.126 |
| Caucasian             | 4400/5395       | 0.94(0.71,1.23) | 0.006  | 69.7          | 0.002 | 70.4         | 0.001 | 70.5         | 0.005 |
| Source of controls    |                 |           |           |              |        |              |        |              |       |
| Population based      | 11123/12811     | 0.87(0.77,0.98) | 0.009  | 46.7          | 0.007 | 50.0         | 0.002 | 54.8         | 0.024 |
| Hospital based        | 1540/1928       | 0.65(0.53,0.79) | 0.111  | 50            | 0.086 | 0.78(0.67,0.92) | 0.585 | 0.74(0.63,0.87) | 0.092 |

*P value of Q-test for heterogeneity test.

Risk in the Asian population (CC vs. GG: OR = 0.80, 95% CI: 0.67–0.96, P = 0.000; GC vs. GG: OR = 0.91, 95% CI: 0.84–0.98, P = 0.139; CG+GC vs. GG: OR = 0.88, 95% CI: 0.79–0.99, P = 0.002; CC vs. GG+GC: OR = 0.86, 95% CI: 0.76–0.98, P = 0.000) but not in the Caucasian population. A decreased risk was also observed for the comparison of CC vs. GG in both studies based population (OR = 0.87, 95% CI: 0.77–0.98, P = 0.000) and hospital based controls (OR = 0.65, 95% CI: 0.55–0.79, P = 0.000) when performed subgroup analysis by the source of controls. In contrast, an increased risk was also observed in the other cancers group for the comparison of CC+GC vs. GG (OR = 1.05, 95% CI: 1.00–1.19, Z = 2.02, P = 0.043, P = 0.222) as summarized in Table 3.

For the rs3746444 polymorphism, there was no significant risk association observed for the overall pooled analysis of cancer risk. However, increased risks were observed for GG vs. AA (OR = 1.23, 95% CI: 1.00–1.50, Z = 2.00, P = 0.045, P = 0.118) and GA vs. AA (OR = 1.19, 95% CI: 1.01–1.41, P = 0.001) and CG+GC vs. GG (OR = 1.14, 95% CI: 1.05–1.25, P = 0.003) in the Asian population rather than in the Caucasian population summarized in Table 4. For the rs2292832, there was no significant association observed in all comparisons (data not shown).

Test of heterogeneity

There was significant heterogeneity across the studies of the rs11614913, rs2910164, rs3746444, and thus the source of heterogeneity was further explored by the heterozygote comparison. For the rs11614913, cancer type (y² = 23.68, df = 5, P = 0.000) and source of control (y² = 5.63, df = 1, P = 0.018) were the source of the heterogeneity. For the rs2910164 polymorphism, cancer type (y² = 27.65, df = 6, P = 0.000) and ethnicity (y² = 15.52, df = 3, P = 0.000) contributed substantially to the heterogeneity. For the rs3746444 polymorphism, ethnicity (y² = 8.38, df = 1, P = 0.004) contributed substantially to heterogeneity.

Sensitivity analysis revealed that the four independent studies [14,15,16,17] were the main cause of heterogeneity for the rs11614913. Heterogeneity was decreased when these studies were removed (TT+CT vs. CC: P = 0.061, F = 33.49%). Similarly, heterogeneity of the rs2910164 (CC+GC vs. GG: P = 0.060, F = 33.5%) and the rs3746444 (GG+GA vs. AA: P = 0.092, F = 39.3%) were decreased when the four [18,19,20,21] and the three [16,22,23] independent studies removed, respectively.

Publication bias

Begg’s funnel plot and Egger’s test were performed to assess the publication bias of the currently available literature. The shape of the funnel plots did not reveal any evidence of obvious asymmetry in all comparison models. Then, the Egger’s test was used to provide statistical evidence for funnel plot symmetry. The results also did not show any evidence of publication bias (rs11614913: t = -0.25, P = 0.086, rs2910164: t = -0.70, P = 0.489, rs3746444: t = 1.88, P = 0.087, rs2292832: t = 1.14, P = 0.318 for dominant model. Figure 1).

Discussion

In this meta-analysis, an association between the four common SNPs in microRNAs (rs11614913, rs2910164, rs3746444, and rs2292832) and cancer risk was evaluated by the pooled results from 40 published studies. The results demonstrated that the rs11614913TT genotype was associated with a decreased risk for developing cancer, in particular for colorectal cancer and lung cancer, or in the Asian population, and that the rs2910164C allele was associated with a decreased risk for developing esophageal cancer, cervical cancer, prostate cancer and HCC, in particular in the Asian population. Contrary to the above, the rs3746444G allele was observed as a risk factor for cancer in the Asian population rather than in the Caucasian population.
population; however, the rs2292832 polymorphism was not associated with cancer risk. The rs11614913 polymorphism present in the miR-196a2 has significantly greater impact on miR-196a expression and is associated with various carcinogenesis [24, 25, 26]. Although there were studies reporting no direct association between rs11614913 and risk of cancers [7, 27, 28, 29], this updated meta-analysis further supported the rs11614913 TT genotype was associated with a decreased risk for cancer. In addition, significant associations were observed in the Asian population but not in the Caucasian population, suggesting a possible ethnic difference in the genetic background and the environment, which was the similar to that reported by Chu et al [28] and Wang et al [27]. In contrast to the published pooled results, this updated pooled results revealed that the rs11614913 TT could be a protective factor against colorectal cancer and lung cancer. However, no significant association was observed in breast cancer, suggesting that carcinogenic mechanisms may differ in the tumor sites and hsa-miR-196a2 genetic variants. The risk of different cancer types should be confirmed by more studies.

For the rs2910164, no significant association was observed in overall pooled results, as supported by the report by Xu et al [7]. In contrast to the published results, this study revealed the different association between rs2910164 polymorphism and cancer risk

### Table 3. Stratification analyses of genetic susceptibility of rs2910164 polymorphism to cancer risk.

| Category | cases/controls | CC vs. GG | GC vs. GG | CC+GC vs. GG | CC vs. GG+GC |
|----------|----------------|-----------|-----------|-------------|--------------|
|          |                | OR(95% CI) | P         | OR(95% CI) | P            | OR(95% CI) | P   | OR(95% CI) | P   |
| Total    |                | 0.88(0.75,1.03) | 0.68 | 0.98(0.90,1.06) | 0.005 | 0.94(0.86,1.02) | 0.58 | 0.91(0.81,1.02) | 0.63 |
| Cancer types |                |            |          |            |          |            |     |            |     |
| HCC      |                | 0.76(0.59,0.99) | 0.313 | 0.92(0.70,1.21) | 0.208 | 0.87(0.71,1.07) | 0.169 | 0.88(0.74,1.05) | 0.371 |
| Gastric cancer |            | 0.92(0.63,1.34) | 0     | 0.96(0.79,1.16) | 0.136 | 0.96(0.74,1.24) | 0.111 | 0.92(0.70,1.21) | 0.835 |
| Breast cancer |            | 1.11(0.93,1.33) | 0.497 | 1.01(0.90,1.11) | 0.538 | 1.03(0.93,1.14) | 0.587 | 1.06(0.92,1.23) | 0.331 |
| Prostate cancer |            | 0.77(0.65,0.91) | 0.425 | 0.90(0.58,1.41) | 0.131 | 0.97(0.92,1.02) | 0.062 | 0.65(0.44,0.96) | 0.699 |
| Cervical cancer |            | 0.50(0.37,0.68) | 0.814 | 0.71(0.51,0.99) | 0.254 | 0.82(0.65,1.04) | 0.382 | 0.65(0.72,1.11) | 0.359 |
| Esophageal cancer |            | 0.58(0.37,0.90) | 0.055 | 0.79(0.65,0.96) | 0.195 | 0.64(0.41,0.98) | 0.079 | 0.67(0.2-1.11) | 0.655 |
| Other cancers |            | 1.06(0.81,1.40) | 0.021 | 2.07(0.94,1.12) | 0.05  | 1.09(1.00,1.19) | 0.222 | 1.03(0.77,1.36) | 0.003 |

**Table 4. Stratification analyses of genetic susceptibility of rs3746444 polymorphism to cancer risk.**

| Category | cases/controls | GG vs. AA | GA vs. AA | GG+GA vs. AA | GG vs. GA+AA |
|----------|----------------|-----------|-----------|--------------|--------------|
|          |                | OR(95% CI) | P         | OR(95% CI) | P            | OR(95% CI) | P   | OR(95% CI) | P   |
| Total    |                | 1.11(0.95,1.29) | 0.127 | 1.12(0.97,1.29) | 0 | 69.8 | 1.12(0.98,1.28) | 0 | 68.2 | 1.06(0.91,1.23) | 0.07 |
| Cancer types |                |            |          |            |          |            |     |            |     |
| HCC      |                | 1.25(0.36,4.34) | 0.023 | 73.6 | 1.00(0.76,1.31) | 0.074 | 61.6 | 1.12(0.63,1.99) | 0.009 | 78.8 | 1.51(0.87,2.56) | 0.06 |
| Breast cancer |            | 1.26(0.70,2.26) | 0.036 | 77.2 | 1.07(0.95,1.20) | 0.163 | 48.6 | 1.08(0.97,1.20) | 0.056 | 72.7 | 1.11(0.87,1.42) | 0.05 |
| Other cancers |            | 1.06(0.85,1.28) | 0.795 | 1.17(0.94,1.46) | 0 | 78.4 | 1.14(0.95,1.36) | 0.001 | 71.2 | 0.98(0.80,1.20) | 0.31 |

**P** value of **Q**-test for heterogeneity test.

**Notes:** Random-effects model was used when a **P** value < 0.05 for heterogeneity test; otherwise, fixed-effects model was used.

| **P** value of **Q**-test for heterogeneity test. | Random-effects model was used when a **P** value < 0.05 for heterogeneity test; otherwise, fixed-effects model was used. |
| 0-25, no heterogeneity; 25-50, modest heterogeneity; 50, high heterogeneity. | 0-25, no heterogeneity; 25-50, modest heterogeneity; 50, high heterogeneity. |
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among ethnicity and the cancer types. The rs2910164 CC genotype was associated with decreased risk for esophageal cancer, cervical cancer, prostate cancer, and HCC in the Asian population, suggesting a difference in genetic background and the environment, and pathogenesis of different tumor sites. The rs2910164 in the miR-146aG
C gene is located in the stem region opposite to the mature miR-146 sequence and results in a change from G:U pair to C:U mismatch in the stem structure of miR-146a precursor. It has been reported that the G-allelic miR-146a precursor could increase the production of mature miR-146a and affecting target mRNA binding [18,19].

The rs3746444 polymorphism present in the miR-499 would target to SOX6 and Rod1 genes important roles for the etiology of cancers [30,31]. The pooled results from 13 studies revealed that rs3746444G allele was associated with an increased risk for developing cancer in the Asian population. To our knowledge, this is the first meta-analysis about the association of rs3746444 of cancer from 11 Asian population studies and two Caucasian population studies. More studies should be accumulated to confirm the results. The rs2292832 polymorphism has also been evaluated by six enrolled studies, with no significant associations were found from all pooled results. Thus far, few epidemiologic studies have investigated the association of rs2292832 polymorphism and cancer risk.

The heterogeneity were observed across the studies for the polymorphisms of rs11614913, rs2910164, rs3746444, the source of the heterogeneity were mainly from the cancer type, such as glioma, gallbladder, bladder, and papillary thyroid carcinoma and cervical cancer, suggesting polymorphisms in miRNAs may play different roles according the cancer type. Furthermore, different risk of polymorphisms in miRNAs was also the source of the heterogeneity, significant associations were observed in the most studies for Asian populations. The studies based on different source of control were also the source of the heterogeneity of studies.

Although meta-analysis is robust, our study still has some limitations. First, our meta-analysis did not evaluate any potential gene-gene interaction and gene-environment interaction due to lack of relevant published data. Second, although all eligible studies were summarized, the relatively small sample size of studies may lead to reduced statistical power when stratified according to the tumor type, ethnicity or infection status. Last, relatively large heterogeneity was observed across the all studies involved.

In summary, this meta-analysis suggested that the rs11614913TT genotype was associated with a decreased cancer risk, especially for colorectal cancer and lung cancer, that the rs2910164C allele was a protective factor for esophageal cancer, cervical cancer, prostate cancer and HCC, and that the rs11614913, rs2910164, and rs3746444 SNPs were risk factors for cancer in the Asian population.
Supporting Information

Figure S1 Process of study selection of case–control studies. (DOC)

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References

1. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116: 281–297.
2. Beraza G, Guayre V, van de Beld J, Wierholds E, Plasterk RH, et al. (2005) Phylogenetice shadowing and computational identification of human microRNA genes. Cell 120: 21–24.
3. Calin GA, Croce CM (2006) MicroRNA signatures in human cancers. Nat Rev Cancer 6: 857–866.
4. Cho WC (2010) MicroRNAs: potential biomarkers for cancer diagnosis, prognosis and targets for therapy. Int J Biochem Cell Biol 42: 1273–1281.
5. Cho WC. (2010) Recent progress in genetic variants and cancer and their implications in diagnostics development. Expert Rev Mol Diagn 10: 699–703.
6. Ryan BM, Robles AI, Harris CC (2010) Genetic variation in microRNA networks: the implications for cancer research. Nat Rev Cancer 10: 399–402.
7. Xu W, Xu J, Liu S, Chen B, Wang X, et al. (2011) Effects of common polymorphisms rs11614913 in miR-196a2 and rs2910164 in miR-146a on cancer susceptibility: a meta-analysis. PLoS One 6: e20471.
8. Honkawak Y, Wood GC, Yang H, Zhao H, Yi Y, et al. (2008) Single nucleotide polymorphisms of microRNA machinery genes modify the risk of renal cell carcinoma. Clin Cancer Res 14: 7956–7962.
9. Christensen BC, Avisar-Whiting M, Ouellet LG, Butler RA, Nelson HH, et al. (2010) Mature microRNA sequence polymorphism in MIR196A2 is associated with risk and prognosis of head and neck cancer. Clin Cancer Res 16: 3713–3720.
10. Handoll HH (2006) Systematic reviews on rehabilitation interventions. Arch Phys Med Rehabil 87: 375.
11. Mudgee AS, Wong JB, Beshansky JR, Porath A, Fleming C, et al. (1994) Cost-effectiveness of streptokinase for acute myocardial infarction. Med Decis Making 14: 108–117.
12. Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ 315: 629–634.
13. Hoffman AE, Zheng T, Yi C, Leaderer D, Weidhaas J, et al. (2009) microRNAs and risk of gallbladder cancer in North Indian population. Mol Biol Rep 38: 1609–1615.
14. Chu H, Wang M, Shi D, Ma L, Zhang Z, et al. (2010) Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid cancer. Proc Natl Acad Sci U S A 105: 7209–7214.
15. Srivastava K, Murray EL, Franssila K, Jarzab B, Schoenberg DR, et al. (2008) Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc Natl Acad Sci U S A 105: 7209–7214.
16. Mittal RD, Gangwar R, George GP, Mittal T, Kapoor R (2011) Investigative role of pre-microRNAs in bladder cancer patients: a case-control study in North India. DNA Cell Biol 30: 401–406.
17. Xu T, Zhu Y, Wei QK, Yuan Y, Zhou F, et al. (2008) A functional polymorphism in the mir-R146a gene is associated with the risk for hepatocellular carcinoma. Carcinogenesis 29: 2126–2131.
18. Zhou F, Zhu H, Luo D, Wang M, Dong X, et al. (2012) A Functional Polymorphism in Pre-miR-146a Is Associated with Susceptibility to Gastric Cancer in a Chinese Population. Cancer Biol Rep 39: 7019–7023.
19. Zhou B, Wang K, Yang Y, Xi M, Zhang Z, et al. (2011) Common genetic polymorphisms in pre-microRNAs and risk of cervical squamous cell carcinoma. Mol Carcinog 50: 499–505.
20. Okubo M, Tahara T, Shibata T, Yamashita H, Nakamura M, et al. (2010) Association between common genetic variants in pre-microRNAs and gastric cancer risk in Japanese population. Helicobacter 15: 524–531.
21. Zhou F, Zhu H, Luo D, Wang M, Dong X, et al. (2012) A Functional Polymorphism in Pre-miR-146a Is Associated with Susceptibility to Gastric Cancer in a Chinese Population. DNA Cell Biol 31: 1290–1293.
22. Zhou B, Wang K, Yang Y, Xi M, Zhang Z, et al. (2011) Common genetic polymorphisms in pre-microRNAs and risk of cervical squamous cell carcinoma. Mol Carcinog 50: 499–505.
23. Yang Y, Fan S, Cao J, Huang S, Zhang LP (2012) Association of the microRNA-1999 variants with susceptibility to hepatocellular carcinoma in a Chinese population. Mol Biol Rep 39: 7019–7023.
24. Hu Z, Chen J, Tian Z, Zhou X, Gu H, et al. (2008) Genetic variants of miRNA sequences and non-small cell lung cancer survival. J Clin Invest 118: 2600–2608.
25. Zhan JF, Chen LH, Chen ZX, Yuan YW, Xie GZ, et al. (2011) A functional variant in microRNA-196a2 is associated with susceptibility of colorectal cancer in a Chinese population. Arch Med Res 42: 144–149.
26. Li XD, Li ZG, Song XX, Liu CF (2010) A variant in microRNA-196a2 is associated with susceptibility to hepatocellular carcinoma in Chinese patients with cirrhosis. Pathology 42: 669–673.
27. Wang F, Ma YL, Zhang P, Yang J, Chen HQ, et al. (2012) A genetic variant in microRNA-196a2 is associated with increased cancer risk: a meta-analysis. Mol Biol Rep 39: 269–275.
28. Jazdzewski K, Murray EL, Franssila K, Jarzab B, Schoenberg DR, et al. (2008) Associated common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc Natl Acad Sci U S A 105: 7209–7214.
29. Qi P, Dou TH, Geng L, Zhou FG, Gu X, et al. (2010) Association of a variant in MIR 196A2 with susceptibility to hepatocellular carcinoma in Chinese patients with chronic hepatitis B virus infection. Hum Immunol 71: 621–626.
30. Okubo M, Tahara T, Shibata T, Yamashita H, Nakamura M, et al. (2010) Association between common genetic variants in pre-microRNAs and gastric cancer risk in Japanese population. Helicobacter 15: 524–531.
31. Tano K, Mizuno R, Okada T, Rakwal R, Shibato J, et al. (2010) MALAT-1 enhances cell motility of lung adenocarcinoma cells by influencing the expression of motility-related genes. FEBS Lett 584: 4575–4580.

Author Contributions

Conceived and designed the experiments: BSH YQ. Performed the experiments: YQX ZLN LPC GQS TYG RL. Analyzed the data: BSH YQX. Contributed reagents/materials/analysis tools: LG. Wrote the paper: BSH WCC SKW.