Research Article

Lack of association between miR-146a rs2910164 C/G locus and colorectal cancer: from a case–control study to a meta-analysis

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Previous studies suggested that miR-146a rs2910164 (C/G) locus was predicted to influence the risk of cancer. However, the relationship of miR-146a rs2910164 locus with colorectal cancer (CRC) susceptibility was controversial. We recruited 1003 CRC patients and 1303 controls, and performed a case–control study to clarify the correlation of miR-146a rs2910164 locus with CRC risk. Subsequently, a comprehensive meta-analysis was conducted to verify our findings. In the case–control study, we suggested that miR-146a rs2910164 variants did not alter CRC risk (CG vs. CC: adjusted \( P = 0.465 \); GG vs. CC: adjusted \( P = 0.436 \), CG/GG vs. CC: adjusted \( P = 0.387 \) and GG vs. CC/CG: adjusted \( P = 0.589 \)), even in subgroup analysis. Next, we conducted a pooled-analysis to identify the correlation of miR-146a rs2910164 locus with CRC risk. In this pooled-analysis, 7947 CRC cases and 12,168 controls were included. We found that miR-146a rs2910164 polymorphism did not influence the risk of CRC (G vs. C: \( P = 0.537 \); GG vs. CC: \( P = 0.517 \), CG/GG vs. CC: \( P = 0.520 \) and GG vs. CC/CG: \( P = 0.167 \)). Our findings suggest that miR-146a rs2910164 C/G polymorphism is not correlated with the susceptibility of CRC. In the future, more case–control studies are needed to confirm our results.

Introduction

In 2018, it was reported that over 1.8 million new colorectal cancer (CRC) cases were diagnosed and 881,000 CRC-related deaths occurred worldwide [1]. In China, CRC ranked both fifth in terms of malignancy incidence and mortality [2]. Trend of cancer incidence indicated that a significant upward trend was found for CRC in developing countries [3]. Previous studies attributed CRC to aging, unhealthy lifestyle and environmental factors [4–7]. It should be very important to understand susceptibility factors of CRC, as they could be beneficial for the evaluation of prevention strategies and susceptibility. Accumulating evidences have shown that an individual’s inherited factors also contribute to the development of CRC.

MicroRNA (miR-) involves ∼22 nucleotides. Recently, miRs have been widely investigated and accepted to be implicated in a number of human diseases, especially in malignancy. Adami et al. indicated that miR-146a was overexpressed in primary stomach tumor tissues without the status of chronic Helicobacter pylori infection and expressed a related lower level in progressed gastric cancer [8]. In addition, a recent study reported that miR-146a expression level decreased in progressed CRC patients with lymph node metastasis [9]. Thus, miR-146a was considered to be implicated in the development and progression of cancer [10,11].

In the past decade, several studies have focused on the correlation of miR-146a rs2910164 C/G with various malignancy susceptibility. MiR-146a rs2910164 C/G locus was suggested to be relevant to an increase susceptibility of CRC in several studies [12,13]. However, two publications suggested that miR-146a rs2910164 G allele had a lower susceptibility of CRC compared to those with C allele [14,15]. Notably,
several pooled-analyses were performed regarding the relationship of CRC risk with this polymorphism [16–23]. However, the previous observations were diversity or even conflicting. Rong et al. and Liu et al. conducted meta-analyses and found that miR-146a rs2910164 polymorphism may confer risk to CRC [17,19]. Other meta-analyses reported that miR-146a rs2910164 was not related to CRC [16,18,20–23]. The controversial findings might be due to the limited sample sizes. The aim of the present study was to explore the potential correlation of miR-146a rs2910164 polymorphism with CRC risk more extensively. We first conducted a case–control study focusing on the correlation of miR-146a rs2910164 with the risk to CRC. Subsequently, we carried out a meta-analysis to further determine the relationship between miR-146a rs2910164 locus and CRC risk.

**Materials and methods**

**Case–control study**

We recruited 1003 sporadic CRC patients between October 2014 and August 2017. They were diagnosed via pathology in Fujian and Jiangsu provinces (China). The mean age of CRC patients was 61.10 ± 12.17 years. In addition, 1303 healthy subjects with a mean age of 61.40 ± 9.61 years were included as controls. The CRC cases and controls were matched by sex and age. The detailed information have been presented in our previous study [24–27]. After giving a written consent in the present study, all participants were questioned the information of risk factor and individual history. The Ethics Committee of Jiangsu University approved the protocol of the present study.

Ethylenediamine tetraacetic acid-anticoagulated blood sample was stored in a −80°C refrigerator. Genomic DNA was isolated from lymphocytes by using a DNA Kit (Promega, Madison, U.S.A.). MiR-146a rs2910164 variants were analyzed by a SNPscan Kit. To confirm the obtained results, 4% DNA samples were randomly selected and reciprocally tested by another person. The reproducibility of genotyping was 100%.

Genotype frequency of miR-146a rs2910164 variants and risk factors were compared by using χ² test. Crude and adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated to predict the association of the miR-146a rs2910164 variants with risk to CRC. A P < 0.05 (two-tailed) was used to determine the significance. Hardy–Weinberg equilibrium (HWE) was used to confirm whether the distribution of miR-146a rs2910164 variants accorded with Mendelian inheritance patterns by a Chi-square software. All the comparisons were conducted using SAS 9.4 version software (SAS Institute, Cary, U.S.A.).

**Meta-analysis**

To verify the correlation between miR-146a rs2910164 locus and the risk of CRC, we subsequently carried out a systematic review and meta-analysis. All studies focusing on the association of miR-146a rs2910164 polymorphism with CRC were identified by a widely internet-based search of China Biology Medicine (CBM), PubMed and Embase databases (published up to March 31, 2019) with the terms of: (miR-146a or hsa-mir-146a or miRNA-146a or MicroRNA-146a or rs2910164) and (colorectal or colon or rectum) and (polymorphism or SNP or variant) and (cancer or carcinoma). Other potential papers were obtained by searching of the references of in eligible publications.

The following included criteria were used: (a) studies based on the association between miR-146a rs2910164 and CRC risk; (b) genotype data were presented; and (c) the genotype distribution in controls consistent with HWE. Two authors (S. Zhang and J. Jiang) extracted the following information independently: the surname of first author, year of publication, country, ethnicity of participant, the region of CRC, method of genotyping and frequencies of authors (S. Zhang and J. Jiang). By using the STATA software (Version 12.0; Stata Corporation, College Station, Texas). A P values (two-sided) less than 0.05 was defined as statistically significant association between miR-146a rs2910164 and CRC risk. By using Power and Sample Size Calculator software [31], power value (α = 0.05) of this meta-analysis was calculated.
Table 1 Distribution of selected characteristics in CRC cases and controls

| Variable                  | Cases (n=1003) | Controls (n=1303) | P     |
|---------------------------|----------------|-------------------|-------|
| Age (years ± standard deviation) | 61.10 ± 12.17 | 61.40 ± 9.61      | 0.496*|
| Age (years)               |                |                   |       |
| <61                       | 451            | 600               | 0.605†|
| ≥61                       | 552            | 703               |       |
| Sex                       |                |                   |       |
| Male                      | 620            | 801               | 0.867†|
| Female                    | 383            | 502               |       |
| Smoking                   |                |                   |       |
| Male                      | 259            | 265               |       |
| Female                    | 383            | 502               |       |
| Alcohol use               |                |                   |       |
| Male                      | 174            | 136               |       |
| Female                    | 300            | 407               |       |
| BMI (kg/m²)               |                |                   |       |
| <24                       | 670            | 688               | <0.001†|
| ≥24                       | 333            | 615               |       |
| Site of tumor             |                |                   |       |
| Colon cancer              | 431            | 42.97             |       |
| Rectum cancer             | 572            | 57.03             |       |

*Two-sided student t test; †Two-sided χ² test
Bold values are statistically significant (P<0.05).
BMI, body mass index

Table 2 Primary information for MiR-146a rs2910164 C/G polymorphism

| Genotyped SNPs | MiR-146a rs2910164 C/G |
|----------------|-----------------------|
| Chromosome     | 5                     |
| Function       | non_coding_transcript_variant |
| Chr Pos (NCBI Build 38) | 160485411 |
| MAF* for Chinese in database | 0.430 |
| MAF in our controls | 0.372 |
| P value for HWE† test in our controls | 0.706 |
| Genotyping method | SNPscan |
| % Genotyping value | 98.87% |

*MAF: minor allele frequency
†http://gvs.gs.washington.edu/GVS147/
‡HWE, Hardy–Weinberg equilibrium

Results
Case–control study
This case–control study consisted of participants enrolled in a study conducted in Jiangsu and Fujian provinces (China) from October 2014 to August 2017. The detailed information of the present study was described in our previous studies [24,25]. Briefly, patients with 431 colon cancer and 572 rectum cancer were recruited. CRC cases were unrelated and confirmed histologically. Non-cancer subjects were included as controls from the same populations with age and sex matched. All subjects were unrelated Chinese Han populations. Table 1 lists demographic information and risk factors that were collected in the present study. Table 2 summarizes the corresponding information of miR-146a rs2910164. The distribution of miR-146a rs2910164 variants in control group is consistent with HWE (P=0.706). Data of genotypes and environmental factors are summarized in Supplementary Table S1.

Table 3 shows the genotype distribution of miR-146a rs2910164 in two groups. MiR-146a rs2910164 variant frequencies were 37.55% (CC), 47.96% (CG) and 14.49% (GG) in CRC patients and 39.69% (CC), 46.23% (CG) and 14.08% (GG) in controls. In the studied populations, minor allele frequency (MAF) of miR-146a rs2910164 C/G polymorphism was 0.372, which was similar to SNP database for Chinese populations (MAF = 0.430, Table 2). We found that there were no significant association between miR-146a rs2910164 polymorphism and susceptibility of
Table 3 Logistic regression analyses of associations between MiR-146a rs2910164 C/G A polymorphism and risk of CRC

| Genotype | Cases (n=1003) | Controls (n=1303) | Crude OR (95%CI) | P | Adjusted OR* (95%CI) | P |
|----------|----------------|-------------------|-----------------|---|---------------------|---|
| MiR-146a rs2910164 | | | | | | |
| CC       | 368            | 37.55             | 516             | 39.69 | 1.00              | 1.00 |
|          | 470            | 47.96             | 601             | 46.23 | 1.10 (0.92–1.31) | 0.317 |
|          | 142            | 14.49             | 183             | 14.08 | 1.09 (0.84–1.30) | 0.300 |
|          | 838            | 85.51             | 1,117           | 85.92 | 1.09 (0.92–1.30) | 0.300 |
|          | 612            | 62.45             | 784             | 60.81 | 1.09 (0.92–1.30) | 0.300 |
|          | 368            | 37.55             | 516             | 39.69 | 1.00              | 1.00 |
|          | 470            | 47.96             | 601             | 46.23 | 1.10 (0.92–1.31) | 0.317 |
|          | 142            | 14.49             | 183             | 14.08 | 1.09 (0.84–1.30) | 0.300 |
|          | 838            | 85.51             | 1,117           | 85.92 | 1.09 (0.92–1.30) | 0.300 |

*Adjusted for age, sex, BMI, alcohol use and smoking status.

CRC (CG vs. CC: crude P=0.317; GG vs. CC: crude P=0.520, CG/GG vs. CC: crude P=0.300; GG vs. CC/CG: crude P=0.780). Adjustments for selected risk factors (e.g. age, sex, BMI, smoking and drinking), the null association was also found (CG vs. CC: adjusted P=0.465; GG vs. CC: adjusted P=0.436, CG/GG vs. CC: adjusted P=0.387 and GG vs. CC/CG: adjusted P=0.589, Table 3).

In subgroup analysis, we found that miR-146a rs2910164 variants did not alter the susceptibility of colon cancer (CG vs. CC: adjusted P=0.863; GG vs. CC: adjusted P=0.880, CG/GG vs. CC: adjusted P=0.846 and GG vs. CC/CG: adjusted P=0.926; Table 4) or rectal cancer (CG vs. CC: adjusted P=0.420; GG vs. CC: adjusted P=0.337, CG/GG vs. CC: adjusted P=0.324 and GG vs. CC/CG: adjusted P=0.484; Table 4).

Meta-analysis of MiR-146a rs2910164 locus and CRC

Next, we conducted a pooled-analysis to identify the correlation of miR-146a rs2910164 with CRC risk. At first, 96 abstracts were collected by searching CBM, EMBASE and Pubmed databases. Figure 1 summarizes the process of meta-analysis. Some publications contained stratified analysis, we treated these subgroups separately [14,32–34]. Table 5 shows the characteristics and miR-146a rs2910164 genotypes of included case-control studies. Finally, 7947 CRC cases and 12,168 controls were included to analyze the relationship of miR-146a rs2910164 with CRC risk [12–15,32–41].

We found that there was no significant association between miR-146a rs2910164 polymorphism and the risk of CRC (G vs. C: P=0.537; GG vs. CC: P=0.517, CG/GG vs. CC: P=0.520 and GG vs. CC/CG: P=0.167; Table 6 and Figure 2). Additionally, subgroup analyses were performed by the ethnicity and region of CRC. Null association between miR-146a rs2910164 and the susceptibility of CRC was also found (Table 6).

In this analysis, publication bias was assessed by using Begg’s test and Egger’s linear regression test. And no significant bias was found in any genetic model (Begg’s test: G vs. C: P=0.820; GG vs. CC: P=0.649; GG/GG vs. CC: P=0.649; GG vs. CC/CG: P=0.381; Egger’s linear regression test: G vs. C: P=0.980; GG vs. CC: P=0.761; GG/GG vs. CC: P=0.686; GG vs. CC/CG: P=0.215; Figure 3).

We used one-way method to evaluate the sensitivity of this analysis. The results suggested that any individual study could not materially alter the association between miR-146a rs2910164 and CRC risk (Figure 4).

Since significant heterogeneity among the studies was found, we conducted subgroup analyses and Galbraith radial plot to identify the source of heterogeneity in a dominant model. Because ethnicity and type of CRC could affect the observations of meta-analysis, subgroup analyses were carried out (Table 6). We found that rectal cancer and Asians might lead to major source of heterogeneity. In Galbraith radial plot, we could find five outliers [12,13,35–37], which contributed significant heterogeneity to this analysis (Figure 5).

NOS scores of the included studies were summarized in Table 5. According to the criterion mentioned in previous study [42,43], we found that only two investigations were poor quality studies [36,44]. When we omitted these poor quality investigations, the findings were not changed.

For this pooled-analysis, the power value (α = 0.05) was 0.258 in G vs. C, 0.235 in GG vs. CC, 0.275 in GG/GG vs. CC and 0.579 in GG vs. CC/CG genetic model.
Table 4 Stratified analyses between MiR-146a rs2910164 polymorphism and CRC risk by site of tumor

| Genotype   | Controls (n=1303) | Colon cancer cases (n=431) | Crude OR (95%CI) | P   | Adjusted OR* (95%CI) | P * | Rectum cancer cases (n=572) | Crude OR (95%CI) | Adjusted OR* (95%CI) | P * |
|------------|-------------------|---------------------------|------------------|-----|---------------------|-----|------------------------|-----------------|----------------------|-----|
|            | n     | %      | n    | %      |       |       | n     | %      |       |       |
| MiR-146a   |       |        |      |        |       |       |       |        |       |       |
| rs2910164  |       |        |      |        |       |       |       |        |       |       |
| CC         | 516   | 39.69  | 165  | 39.01  | 1.00  | 1.00  | 203   | 36.45 | 1.00  | 1.00  |
|            |       |        |      |        |       |       |       |        |       |       |
|            |       |        |      |        |       |       |       |        |       |       |
| CG         | 601   | 46.23  | 200  | 47.28  | 1.04  | 0.742 | 1.02  | 0.80–1.30 | 0.863 | 1.02  | 0.82–1.30 | 0.846 |
|            |       |        |      |        |       |       |       |        |       |       |       |
|            |       |        |      |        |       |       |       |        |       |       |       |
| GG         | 183   | 14.08  | 58   | 13.71  | 0.99  | 0.960 | 1.03  | 0.73–1.45 | 0.880 | 1.03  | 0.73–1.45 | 0.848 |
|            |       |        |      |        |       |       |       |        |       |       |       |
|            |       |        |      |        |       |       |       |        |       |       |       |
| GG+CG      | 784   | 60.31  | 258  | 60.99  | 1.03  | 0.803 | 1.02  | 0.82–1.28 | 0.846 | 1.02  | 0.82–1.28 | 0.846 |
|            |       |        |      |        |       |       |       |        |       |       |       |
|            |       |        |      |        |       |       |       |        |       |       |       |
| CC+CG      | 1117  | 85.92  | 365  | 86.29  | 1.00  | 1.00  | 473   | 84.92 | 1.00  | 1.00  | 1.00  | 1.00  |
|            |       |        |      |        |       |       |       |        |       |       |       |
|            |       |        |      |        |       |       |       |        |       |       |       |
| GG         | 183   | 14.08  | 58   | 13.71  | 0.97  | 0.852 | 1.02  | 0.74–1.40 | 0.926 | 1.02  | 0.74–1.40 | 0.926 |
|            |       |        |      |        |       |       |       |        |       |       |       |
|            |       |        |      |        |       |       |       |        |       |       |       |
| G allele   | 967   | 37.19  | 316  | 37.35  | 0.97  | 0.852 | 1.02  | 0.74–1.40 | 0.926 | 1.02  | 0.74–1.40 | 0.926 |

*Adjusted for age, sex, smoking status, alcohol use and BMI status.
Table 5 Characteristics and genotypes of the included studies in meta-analysis for MiR-146a rs2910164 polymorphism

| Study          | Publication year | Ethnicity | Country                | Region of CRC | Sample size (case/control) | Genotype method | Genotype | Case | Control | HWE | NOS |
|----------------|------------------|-----------|------------------------|---------------|-----------------------------|-----------------|----------|------|---------|-----|-----|
| Hezova et al.  | 2012             | Caucasians| Czech Republic         | Mixed         | 197/122                     | Taqman          | GG       | 115  | 70     | 12  | 124| Yes | 7 |
| Min et al.     | 2012             | Asians    | Korea                  | Colon         | 251/502                     | PCR-RFLP        | GG       | 31   | 144    | 76  | 69 | 245| 188| Yes | 7 |
| Min et al.     | 2012             | Asians    | Korea                  | Rectum        | 184/502                     | PCR-RFLP        | CG       | 28   | 87     | 69  | 69 | 245| 188| Yes | 7 |
| Chae et al.    | 2013             | Asians    | Korea                  | Rectum        | 176/568                     | PCR-RFLP        | CC       | 23   | 87     | 66  | 121| 282| 165| Yes | 7 |
| Chae et al.    | 2013             | Asians    | Korea                  | Colon         | 221/568                     | PCR-RFLP        | CC       | 38   | 93     | 90  | 121| 282| 165| Yes | 7 |
| Lv et al.      | 2013             | Asians    | China                  | Mixed         | 353/540                     | PCR-RFLP        | CC       | 54   | 230    | 47  | 96 | 274| 143| Yes | 7 |
| Ma et al.      | 2013             | Asians    | China                  | Rectum        | 588/1,203                   | Taqman          | CG/CC    | 229  | 97     | 122 | 124| 65 | 91 | Yes | 7 |
| Ma et al.      | 2013             | Asian     | China                  | Colon         | 559/1,203                   | Taqman          | CG/CC    | 215  | 97     | 122 | 124| 65 | 91 | Yes | 7 |
| Vinci et al.   | 2013             | Caucasians| Italy                  | Mixed         | 160/160                     | HRM             | GG       | 86   | 13     | 71  | 100| 65 | 13 | Yes | 5 |
| Kupcinskas et  | 2014             | Caucasians| Lithuania and Latvia   | Mixed         | 193/428                     | Taqman          | GG       | 140  | 50     | 2   | 275| 134| 15 | Yes | 7 |
| Mao et al.     | 2014             | Asians    | China                  | Mixed         | 555/566                     | SNPscan         | GG       | 70   | 291    | 186 | 85 | 271| 205| Yes | 9 |
| Hu et al.      | 2014             | Asians    | China                  | Mixed         | 276/373                     | PCR-RFLP        | GG       | 34   | 82     | 84  | 44 | 187| 142| Yes | 7 |
| Dikaiakos et   | 2015             | Caucasians| Greece                | Mixed         | 157/299                     | PCR-RFLP        | GG       | 8    | 48     | 101 | 21 | 120| 158| Yes | 7 |
| Ying et al.    | 2016             | Asians    | China                  | Mixed         | 1358/1079                   | MassARRAY       | GG       | 223  | 655    | 473 | 163| 529| 383| Yes | 6 |
| Zhang et al.   | 2016             | Asians    | China                  | Mixed         | 106/53                      | PCR-RFLP        | GG       | 28   | 62     | 16  | 10 | 25 | 18 | Yes | 6 |
| Lindor et al.  | 2017             | Caucasians| Australia, U.S.A., and Canada | Mixed         | 899/204                     | Not available   | G: 1390 | 408  | C: 320  | Yes | 5 |
| Chayeb et al.  | 2018             | Caucasians| Tunisia               | Rectum        | 57/161                      | PCR-RFLP        | GG       | 20   | 31     | 6   | 49 | 89 | 23 | Yes | 8 |
| Chayeb et al.  | 2018             | Caucasians| Tunisia               | Colon         | 95/161                      | PCR-RFLP        | GG       | 40   | 46     | 9   | 49 | 89 | 23 | Yes | 8 |
| Gao et al.     | 2018             | Asians    | China                  | Mixed         | 560/780                     | PCR-RFLP        | GG       | 130  | 285    | 145 | 149| 411| 220| Yes | 7 |
| Our study      | 2019             | Asians    | China                  | Colon         | 431/1303                    | SNPscan         | GG       | 58   | 200    | 165 | 183| 601| 516| Yes | 6 |
| Our study      | 2019             | Asians    | China                  | Rectum        | 572/1303                    | SNPscan         | GG       | 84   | 270    | 203 | 183| 601| 516| Yes | 6 |

*indicates CG/CC
HWE, Hardy–Weinberg equilibrium
Table 6 Meta-analysis of the MiR-146a rs2910164 polymorphism and CRC risk

| No. of study | G vs. C |      |      | GG vs. CC |      |      | GG+CG vs. CC |      |      | GG vs. CC+CG |      |
|--------------|--------|------|------|----------|------|------|-------------|------|------|--------------|------|
|              | OR (95%CI) | P | (Q-test) | OR (95%CI) | P | (Q-test) | OR (95%CI) | P | (Q-test) | OR (95%CI) | P | (Q-test) |
| Overall      | 21     | 1.03 (0.94–1.12) | 0.537 | <0.001 | 1.06 (0.89–1.27) | 0.517 | 0.002 | 1.05 (0.90–1.24) | 0.520 | <0.001 | 1.08 (0.97–1.20) | 0.167 | 0.033 |
| Type         |        |      |      |          |      |      |              |      |      |              |      |      |
| Colon cancer | 5      | 1.01 (0.80–1.28) | 0.908 | 0.006 | 0.98 (0.65–1.48) | 0.914 | 0.040 | 1.02 (0.69–1.51) | 0.926 | 0.002 | 1.07 (0.84–1.35) | 0.600 | 0.069 |
| Rectal cancer| 5      | 0.98 (0.78–1.22) | 0.823 | 0.021 | 0.95 (0.59–1.51) | 0.815 | 0.024 | 0.97 (0.74–1.28) | 0.853 | 0.083 | 1.05 (0.80–1.36) | 0.741 | 0.037 |
| Mixed type of CRC | 11 | 1.06 (0.95–1.17) | 0.294 | 0.021 | 1.17 (0.92–1.48) | 0.192 | 0.044 | 1.12 (0.87–1.44) | 0.373 | <0.001 | 1.09 (0.97–1.22) | 0.132 | 0.176 |
| Ethnicity    |        |      |      |          |      |      |              |      |      |              |      |      |
| Asians       | 14     | 1.03 (0.94–1.13) | 0.547 | 0.002 | 1.06 (0.88–1.29) | 0.530 | 0.003 | 1.09 (0.92–1.29) | 0.343 | <0.001 | 1.06 (0.93–1.19) | 0.393 | 0.023 |
| Caucasians   | 7      | 1.03 (0.84–1.26) | 0.796 | 0.020 | 1.09 (0.63–1.87) | 0.760 | 0.083 | 0.95 (0.60–1.50) | 0.835 | 0.080 | 1.16 (0.96–1.41) | 0.129 | 0.268 |
| NOS scores   |        |      |      |          |      |      |              |      |      |              |      |      |
| ≥ 6          | 19     | 1.04 (0.95–1.14) | 0.396 | <0.001 | 1.08 (0.90–1.30) | 0.405 | 0.002 | 1.07 (0.91–1.26) | 0.419 | <0.001 | 1.09 (0.97–1.21) | 0.142 | 0.030 |
| < 6          | 2      | 0.91 (0.74–1.12) | 0.368 | 0.712 | 0.66 (0.30–1.43) | 0.291 | – | 0.66 (0.31–1.41) | 0.286 | – | 0.91 (0.59–1.39) | 0.654 | – |

CRC, colorectal cancer;
**Discussion**

In CRC patients, *miR-146a* expression level decreased in progressed tumors [9]. Since *miR-146a* plays important roles in the development of CRC, we presumed that *miR-146a* rs2910164 variants could influence the susceptibility to CRC. Here, we first performed a case–control study and subsequently conducted a meta-analysis on the potential correlation between *miR-146a* rs2910164 polymorphism and the susceptibility of CRC. In the case–control study, we recruited 1003 sporadic CRC patients and 1303 controls. In meta-analysis, the present study included 21 independent studies with 20,115 subjects (7947 CRC patients and 12,168 controls). Nevertheless, null correlation has been identified between *miR-146a* rs2910164 locus and the risk of CRC, even in different subgroup.

To date, some studies have been carried out to assess this potential correlation between *miR-146a* rs2910164 polymorphism and CRC susceptibility in different populations. However, conflicting findings were found. Consequently, we designed a case–control study matching with age and sex. We got a null correlation between this SNP and the risk of CRC. These findings were similar to some previous publications, which the genotype frequency of the *miR-146a* rs2910164 G allele had no significant difference in CRC cases versus non-cancer controls [35,36,39,40]. It was worth mentioning that the paper was published by Ying *et al.* [40], where the investigation included more than 1000 CRC patients and 1000 controls. However, other publications, identified a significant associations or even suggested opposite findings. Dikaiakos *et al.* [15] and Chae *et al.* [14] reported a decreased risk of the *miR-146a* rs2910164 G allele to CRC. Recently, two publications found an association of the increased risk with *miR-146a* rs2910164 G allele [12,13]. It could be interpreted that these investigations were carried out in different ethnicity, region, age and with variable sample size. A recent meta-analysis has found an association for the European populations in the recessive model.
Figure 2. Meta-analysis of the association between MiR-146a rs2910164 polymorphism and CRC risk (GG+CG vs. CC, random-effects model)

Figure 3. Begg’s funnel plot of meta-analysis (GG+CG vs. CC, random-effects model)
Compared with the meta-analysis mentioned above, the current meta-analysis has included more case–control studies with larger sample sizes. Thus, our findings might be more credible.

In the large-scale pooled-analysis, we found that miR-146a rs2910164 might not confer the risk to CRC. However, the interaction between genes and environment factors might be implicated in the occurrence of CRC. Reviewing early publications, some reasons were identified for the conflicting findings. First, different ethnicity may lead to the different results, since the genotype frequencies of the miR-146a rs2910164 were diverse between Asians and Caucasians. For example, in the present study, MAF value of miR-146a rs2910164 (C/G) was 0.404 in Asians, while the value was 0.631 in Caucasians. On the other hand, most of the included case–control studies did not focus on
some vital environmental risk factors (e.g., low intake of dietary fiber, alcohol consumption, tobacco use, obesity, overweight and being physically inactive). It is possible that miR-146a rs2910164 has influence the development of CRC, while those environmental factors mentioned above may cover up the role of C→G variation in rs2910164. Thus, in the future, more case–control studies should be designed to explore the relationship of miR-146a rs2910164 on the risk of CRC, especially focusing on the interaction of gene–environmental factors.

In the present study, heterogeneity might be noted, which could significantly affect the explanation of our findings. When we carried out subgroup analyses, significant heterogeneity was found in colon cancer and Asians subgroups. Combined the Galbraith radial plot (Figure 5) and the forest plot (Figure 2) in a dominant model, we identified five outliers [12,13,35–37], which might contribute to prominent heterogeneity. The quality evaluation was used to improve the premet study. In the meta-analysis, we found that only two investigations were poor quality study [36,44]. In subgroup analysis, when we omitted them, the findings were not altered materially.

There are, whereas, some potential limitations in the present study. First, this case–control study only focused on Chinese Han populations. Second, for lack of parameter of risk factors, we only calculate the crude ORs and CIs to assess the correlation between miR-146a rs2910164 and CRC risk in subsequent meta-analysis. Finally, due to the limited participants in subgroups, the power of subgroup analysis might be insufficient.

To conclude, our findings have not supported a relationship between the miR-146a rs2910164 and risk of CRC. The importance of this locus as a genetic predictor for the development of CRC may be very inappreciable and the significance of hereditary screening in healthy individuals may be lack of evidence. In the future, larger studies with well-matched controls are needed to confirm our findings.

Data Availability
The genotypes and environmental factors are summarized in Supplementary Table S1.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Funding
The project was supported by the Application and Basic Research Project of Changzhou City [grant number CJ20180068].

Author Contribution
Conceived and designed the experiments: Z.Q. Performed the experiments: S.Z., J.J., W.T. Analyzed the data: J.J., W.T. Contributed reagents/materials/analysis tools: Z.Q. Wrote the manuscript: S.Z., J.J.

Acknowledgements
We appreciate all participants who participated in this study. We wish to thank Dr Yan Liu (Genesky Biotechnologies Inc., Shanghai, China) for technical support.

Abbreviations
CI, confidence interval; CRC, colorectal cancer; HWE, Hardy–Weinberg equilibrium; OR, odds ratio.

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