**Introduction**

MicroRNAs (miRNAs) are non-coding RNAs of approximately 21 to 25 nucleotides in length. Mature miRNAs target the 3' untranslated region of mRNA, thereby degrading mRNA or suppressing translation (1,2). A single miRNA can regulate multiple genes implicated...
in multiple pathways (3), and a signaling pathway can be affected by a series of miRNAs (4,5). Therefore, miRNAs have key functions in gene network regulation as well as in physiological and pathological processes, including tumorigenesis, proliferation, apoptosis, and metabolism (1,3,6-8).

MiR-34b and miR-34c share a common primary transcript (pri-miR-34b/c). These miRNAs are frequently downregulated and function as tumor suppressors in several types of cancer. For instance, the miR-34 family members are direct transcriptional targets of p53 and constitute a part of the p53 tumor suppressor network. These family members also regulate cell cycle arrest, apoptosis, and senescence (9,10). Microarray analyses have revealed that the miR-34 family can down-regulate numerous putative target genes, including CDK4/6, Cyclin E2, MET, and Bcl-2, which have important functions in tumor development (9-11). MicroRNA-34b can also inhibit prostate cancer via demethylation, active chromatin modifications, and AKT pathways (12).

Pri-miR-34b/c contains a corresponding promoter. Single nucleotide polymorphisms (SNPs) in the promoter region may be important in the alteration of expression and/or function of mature miR-34b/c and its targeted genes, thereby affecting tumor-related pathways. A potentially functional polymorphism (rs4938723 T/C) has been discovered in the promoter region of pri-miR-34b/c by in silico analysis. The T to C shift of the rs4938723 polymorphism possibly influences GATA-X binding sites. If the polymorphic location is C, then it can bind to the GATA-X; otherwise, it cannot bind to the GATA-X (13). Studies have identified that the genetic variants in the primary miRNA sequence of miR-34b/c (rs4938723 T/C) can be used as possible biomarkers of cancer development. However, inconsistent and inconclusive results have been reported probably because of the slight effect of polymorphism on cancer risk or the relatively small sample size in published studies. Therefore, we conducted this meta-analysis to summarize the effect of the miR-34b/c polymorphism on cancer risk.

Materials and methods

Publication search

We searched the PubMed, Web of Science, and Chinese National Knowledge Infrastructure (CNKI) databases (last updated on May 1th, 2014) for published studies on the relationship of miR-34b/c polymorphism with cancer risk. The following keywords were used either separately or together: “miR-34b/c or rs4938723”; “polymorphism or mutation”; and “cancer or tumor”. The references of related studies were reviewed to search other potentially related articles manually. All of the selected studies in our meta-analysis should also match the following inclusion criteria: (I) human case-control study; (II) evaluation of miR-34b/c rs4938723 polymorphism and cancer risk; and (III) availability of genotype frequencies in the study. We also restricted our search to studies published in English and Chinese. The procedures of database search are shown in Figure 1.

Data extraction

Two investigators independently obtained the following information from each study: (I) name of the first author; (II) year of publication; (III) country of origin; (IV) ethnicity; (V) cancer type; (VI) total number of control subjects and cases; (VII) genotype frequencies for cases and control subjects; and (VIII) Hardy-Weinberg equilibrium (HWE) of control subjects. Differences in the findings of the investigators were resolved by discussion.

Statistical analysis

HWE was calculated for the control subjects of each study by using a Chi-square goodness-of-fit test. Studies with inconsistent HWE (P<0.05) were removed. The STATA
software (version 12.0; Stat Corporation, College Station, Texas, USA) was used to conduct the statistical analysis; all of the tests were two-sided. The odds ratio (OR) and 95% confidence interval (CI) were used to evaluate the strength of the association between the miR-34b/c polymorphism and cancer risk. We determined the relationship between SNP and cancer susceptibility by using the following genetic models: homozygote model (CC/TT); heterozygote model (CT/TT); the dominant genetic model (CCCT/TT); and recessive genetic model (CC/CTTT). Subgroup analyses were also conducted according to cancer type and ethnic group. The Z test was used to determine the significance of the pooled OR, in which the results were considered statistically significant at P<0.05. The heterogeneity among studies was determined by using a Chi-square-based Q test. The fixed-effect model (Mantel-Haenszel model) was used when homogeneity was considered significant (P>0.05); otherwise, the random-effect model (DerSimonian and Laird method) was used. In addition, publication bias was assessed using Begg’s funnel plot and Egger’s test (P<0.05 was considered a significant publication bias). These procedures were conducted twice in our meta-analysis.

Results

Characteristics of the studies

After an extensive search was conducted, a total of ten studies (with 6,036 cancer patients and 6,204 control subjects) satisfied our criteria. The details of each study are shown in Table 1. Among these studies, three focused on hepatocellular carcinoma (HCC), two focused on colorectal cancer (CRC), two focused on breast cancer (BC) and the other three focused on other types of cancers (13-21). The genotypic distributions in the control populations were consistent with HWE in the selected studies.

Quantitative synthesis and heterogeneity analysis

We observed that hsa-miR-34b/c rs4938723 T > C polymorphism was weakly associated with overall cancer susceptibility in the variant CT genotype (Table 2 and Figure 2) after we pooled the selected studies in the meta-analysis. The Q-test of heterogeneity was always significant, and we conducted analyses using random effect models for the overall population if P_{heterogeneity}<0.05. Then, a stratified analysis was performed according to cancer type. The results showed that the variant CT (OR =1.19, 95% CI: 1.03-1.37) and CC/CT (OR =1.18, 95% CI: 1.03-2.35) genotypes were associated with an increased risk of HCC compared with wild-type TT genotype. However, a decreased risk of CRC was found in the genetic model of CC/TT (OR =0.66, 95% CI: 0.47-0.92) and CC/CTTT (OR =0.67, 95% CI: 0.49-0.93) (Figure 2). We also performed the analysis stratified by ethnicity. The results indicated that the variant CT (OR =1.12, 95% CI: 1.02-1.22) genotype were associated with an increased risk of cancer compared with wild-type TT in Asian population (Figure 3). Base on the difference of OR value in subgroups, we thought that different cancer
types were the main origin of heterogeneity.

**Publication bias**

Publication bias was assessed by Begg's funnel plot and Egger's test (Figure 4). We found that the graphical funnel plots were symmetrical for the comparison of the genetic models. Egger's test results did not indicate any evidence of publication bias in our meta-analysis (P>0.05).

**Sensitivity analysis**

Sensitivity analyses were performed after sequential removal of each eligible study. The results suggested that the significance of the pooled ORs in overall analysis was influenced in heterozygote model by Yan Xu et al.'s study (14). The pooled OR and CI values were weakly changed by exclusion of this study: OR =1.09 (95% CI: 0.99-1.20) after removal. Therefore, the positive result about heterozygote model and cancer risk in Figure 2 and Figure 3 and Table 2 was needed to be treated with caution. Based on the above data, lack of association was found between hsa-miR-34b/c rs4938723 polymorphism and overall cancer susceptibility. Further, the sensitivity analysis suggested that the significance of the pooled ORs was not influenced by any single study in other genetic models (data not shown).

**Discussion**

Evidence indicates that a complex network of “miRNA-mRNA” is involved in human cancer (22). However, the mechanisms regulating miRNA expression and function have not been elucidated. SNPs in miRNA genes can possibly affect gene expression and function by modulating the transcription of the primary transcript (pri-miRNA) as well as the processing and maturation of pre-miRNA. Studies have shown that pri-miRNAs or pre-miRNA SNPs are associated with cancer risk. For instance, rs7372209

<table>
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<td>0.67 (0.49-0.93)</td>
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</table>

* Number of comparisons; **, Fix-effects model (F) was used when P value for heterogeneity ≥0.05; otherwise, random-effects model (R) was used; †, P value of Q-test for heterogeneity test. HCC, hepatocellular carcinoma; CRC, colorectal cancer.
in pri-miR-26a-1 is associated with a 64% decreased risk of bladder cancer in females and a twofold increased risk of premalignant oral lesions (23,24). The rs531564 SNP in pri-miR-124-1 is also associated with increased risks of bladder cancer and esophageal cancer (EC) in males (25). Furthermore, the pri-miRNA SNP rs213210 in miR-219 is associated with an increased the risk of EC. pre-miR-196a2 rs11614913 polymorphism is also associated with a significant increased risk of BC (26).

In our meta-analysis, lack of association was found between the hsa-miR-34b/c rs4938723 polymorphism and cancer susceptibility based on ten case-control studies including 6,036 cancer patients and 6,204 control subjects. Subgroup analysis revealed that the variant CT and CC/TT genetic models were associated with an increased risk of HCC compared with wild-type TT genotype. However, a decreased risk of CRC was found in the homozygote genetic model and the recessive genetic model. Recently, CpG methylation of miR-34b/c has been detected in varieties of human cancers. There may be a possibility that...

![Forest plot showing the stratified analysis performed according to cancer type. (A) CC/TT; (B) CT/TT; (C) CCCT/TT; (D) CC/CTTT. The fixed effect model was used.](image-url)
the methylation status of miR-34b/c is different between HCC and CRC, which results in the different effect of the miR-34b/c rs4938723 polymorphism on the risk of HCC and CRC. Further large-scale studies, however, should be done to improve the generalization of the results (13,27). Our meta-analysis showed several limitations. First, cancer is caused by many factors, such as genetic disorders and environmental damage, while we only focused on the function of SNP in this study. Second, cancer patients from the studies of Han et al. and Son et al. (15,19) carried hepatitis B/C virus, which may interact with pre-miR-34b/c and influence the results. Third, the lack of original data, including age, gender, and control source, limited our analysis. Fourth, only studies published in English and Chinese were included in our meta-analysis. This language criterion may restrict our sample size.

Despite these limitations, our meta-analysis reveals that there is no association between hsa-miR-34b/c rs4938723 polymorphism and overall cancer risk based on current studies. Further, this polymorphism plays a different role in the risk of HCC and CRC. To confirm our results, we recommend that future well-designed studies and large sample size should consider diverse ethnic populations and cancer types.
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References


