A comprehensive review of microRNA-related polymorphisms in gastric cancer

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ABSTRACT. MicroRNAs (miRNAs) are a class of small non-coding RNA molecules of about 22 nucleotides in length. miRNAs are highly conserved in both plants and animals, and function as gene regulators by binding to the 3'-untranslated region of target mRNAs for cleavage and/ or translational repression. miRNA biogenesis, stability, and regulation of expression are strongly sequence dependent. Sequence variants, such as single nucleotide polymorphisms (SNPs) in pri-miRNA, pre-miRNA, promoter regions, or miRNA-target sites, can influence miRNA function, thereby contributing to the pathological features of human disease. In this review, we focus on miRNA-related SNPs in gastric cancer and comprehensively analyze some commonly studied SNPs.

Key words: MicroRNA; Polymorphism; Gastric cancer; Review
INTRODUCTION

MicroRNAs (miRNAs) are a class of small non-coding RNA molecules of about 22 nucleotides in length. The first miRNA (lin-4) was discovered by Lee et al. (1993) who reported that lin-4 can control the timing of Caenorhabditis elegans larval development. Subsequently, more than 1000 miRNAs have been identified, which can regulate nearly 30% of human genes. miRNAs are first transcribed by RNA polymerase II in the nucleus, forming primary miRNAs (pri-miRNAs). pri-miRNAs are then cleaved into hairpin loops (70-100 nucleotides) of precursor structure (pre-miRNA) by the Drosha-DGCR8 complex (Lee et al., 2003). pre-miRNAs are transported to the cytoplasm by exportin 5/Ran GTP, where pre-miRNAs are cleaved by the RNase III enzyme Dicer, resulting in mature miRNAs (Lund and Dahlberg, 2006). miRNAs are highly conserved in both plants and animals, and they function as gene regulators by binding to the 3'-untranslated region (3'-UTR) of target mRNAs for cleavage and/or translational repression (Lee et al., 1993).

miRNA biogenesis, stability, and regulation of expression are strongly sequence dependent (Leshkowitz et al., 2013). Sequence variants, such as single nucleotide polymorphisms (SNPs) in pri-miRNA, pre-miRNA, promoter regions, or miRNA target sites, can influence miRNA function, thereby contributing to the pathological features of human disease (Sethupathy et al., 2007). Sethupathy et al. (2007) reported an SNP (rs5186) in the 3'-UTR of angiotensin II receptor type 1 (AGTR1), where the rs5186 C allele abrogates an existing miR-155 binding site and induces elevated expression of AGTR1, which is implicated in hypertension. In view of the potential roles of miRNA-related SNPs, large numbers of studies have been carried out to identify disease-associated miRNA polymorphisms. In this review, we focus on miRNA-related SNPs in gastric cancer and comprehensively analyze some commonly studied SNPs.

MATERIAL AND METHODS

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He et al. (2005) provided the first evidence that miRNA-related polymorphisms can change the expression of miRNAs and their target genes, which may contribute to carcinogenesis. Subsequently, miRNA-related polymorphisms were regarded as a gold mine for molecular epidemiology and a large number of studies have been done to assess the association of genetic polymorphisms with cancer risk. In this study, we review miRNA-related polymorphisms and gastric cancer. We searched PubMed using the following terms: “miRNA”, “polymorphism”, and “gastric cancer” (last search update: April 2, 2015). From this search, 64 records were identified and, after reading the full text, 45 studies reporting the relationship of SNPs with gastric cancer risk were included in this study.

The overview of miRNA-related polymorphisms evaluated in gastric cancer is shown in Table 1. According to SNP location, four groups were formed: pri-miRNA, pre-miRNA, promoter of miRNA, and 3'-UTR of target transcript. Overall, these are involved in more than 100 miRNAs, including miR-146a, miR-196a2, and miR-499a/b/c.
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RESULTS AND DISCUSSION

To date, the most commonly studied polymorphisms are rs2910164, rs11614913, rs2292832, and rs3746444, which are located in pre-miR-146a, pre-miR-196a2, pre-miR-149, and pre-miR-499, respectively. These polymorphisms were first reported by Hu et al. (2008), who found that rs11614913 might be used as prognostic biomarker for non-small cell lung cancer. Regarding the first report of the polymorphism in gastric cancer, Zeng et al. (2010) found that subjects with pre-miR-146a rs2910164 GC/GG genotypes had a 1.58-fold elevated risk of gastric cancer compared to carriers of the CC genotype [95% confidence interval (CI) = 1.11-2.20; P = 0.009]. For SNPs in pri-miRNA, Xu et al. (2014b) reported an rs629367 polymorphism located in pri-let-7a-2 and the CC genotype was associated with an increased risk.
of gastric cancer [odds ratio (OR) = 1.83; 95%CI = 1.00-3.32] and poorer survival [hazard ratio (HR) = 4.48; 95%CI = 1.60-12.60]. Arisawa et al. (2012) reported a polymorphism (rs2505901) in pri-miR-938 and the CC genotype was associated with a decreased risk of gastric cancer (OR = 0.73; 95%CI = 0.55-0.99). Stenholm et al. (2013) reported that the variant allele of rs7372209 in pri-miR-26a1 was associated with a worse prognosis of advanced gastric cancer. In our previous study, we found that a polymorphism (rs4938723) in the promoter of miR-34b/c was associated with a decreased risk of gastric cancer (OR = 0.66; 95%CI = 0.45-0.97) (Pan et al., 2015). Our result was confirmed by another study (Yang et al., 2014). Moreover, Xu et al. (2014a) reported a similarly decreased effect of rs35010275 in the promoter of miR-196a2 on gastric cancer (adjusted OR = 0.85; 95%CI = 0.77-0.94). Besides SNPs in pri-miRNA, pre-miRNA, and promoters of miRNA, SNPs in the 3'-UTR of miRNA target genes have also been studied widely. For example, our other previous study showed that the rs712 polymorphism in the 3'-UTR of let-7 was associated with an increased risk of gastric cancer (adjusted OR = 3.05; 95%CI = 1.53-6.08) (Li et al., 2013b). Li et al. (2014a) reported that the rs12904 polymorphism in the 3'-UTR of miR-200c was significantly associated with risk of gastric cancer (OR = 0.65; 95%CI = 0.50-0.85), and the rs12904 A allele may abrogate miR-200c binding, inducing elevated levels of its target gene ephrin-A1. Lin et al. (2012) reported that the rs12537 CT/TT genotypes in the miR-181a binding site were associated with a significantly increased risk of gastric cancer (adjusted OR = 1.72; 95%CI = 1.36-2.16) and poor overall survival (HR = 1.38; 95%CI = 1.03-1.83). Further analysis showed that the rs12537 CT genotype carriers had lower expression of the miR-181a target gene myotubularin-related protein 3.

Since gastric cancer is a complex disease with multiple factors involved, gene-gene and gene-environment analyses were also evaluated. Xu et al. (2014c) reported an increased interaction effect of the pri-let-7a-1 rs10739971 polymorphism with ERCC6 rs1917799 on gastric cancer (OR = 2.59; 95%CI = 1.12-5.97). Zhang et al. (2012) reported that smokers carrying pre-miR-149 rs2292832 CT/CC genotypes and pre-miR-605 rs2043556 AG/GG genotypes had an increased risk of gastric cancer (OR = 1.87; 95%CI = 1.03-3.42), and tea drinkers with the pre-miR-149 rs2292832 CT/CC genotypes were protected from gastric cancer (OR = 0.47; 95%CI = 0.29-0.77).

Association of the pre-miR-146a rs2910164 polymorphism with risk of gastric cancer

Over the past several years, the association of the pre-miR-146a rs2910164 polymorphism with risk of gastric cancer has been extensively investigated (Okubo et al., 2010; Zeng et al., 2010; Hishida et al., 2011; Wang et al., 2012; Zhou et al., 2012a,b; Ahn et al., 2013; Ma et al., 2013; Stenholm et al., 2013; Wu et al., 2013a; Dikeakos et al., 2014; Kupcinskas et al., 2014; Li et al., 2014b; Parlayan et al., 2014; Pu et al., 2014; Tahara et al., 2014; Xu et al., 2014d, 2015). In total, 18 studies were involved, including 10 case-control studies, 6 meta-analyses, and 2 case-only studies. As shown in Table 2, most of the studies were conducted in Asia, mainly in China, and only 3 studies were done in Europe. In case-control studies, some authors reported a positive result (Okubo et al., 2010; Zeng et al., 2010; Zhou et al., 2012a; Kupcinskas et al., 2014) and others reported a negative result (Hishida et al., 2011; Ahn et al., 2013; Dikeakos et al., 2014; Parlayan et al., 2014). To make the results more precise, some authors performed meta-analyses by combining all the data together (Ma et al.,
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2013; Wu et al., 2013a; Li et al., 2014b; Xu et al., 2014d, 2015), which improved the statistical power. However, conflicting results were still present. For example, Li et al. (2013a) reported that the miR-146a rs2910164 polymorphism was significantly associated with a decreased risk of gastric cancer under the allele model (OR = 0.87; 95%CI = 0.81-0.93) and recessive model (OR = 0.81; 95%CI = 0.72-0.90). Xu et al. (2015) reported that the miR-146a rs2910164 polymorphism was not associated with gastric cancer risk. Although it is difficult to decipher the exact reason for the inconsistent results, sample size, study design, and ethnicity should be considered. If the sample size is not large enough, the power is insufficient and false-negative results may occur. If the study design is hospital-based, selection bias cannot be ruled out and the results may be unreliable. Moreover, it is well known that genetic variants are diverse in different ethnicities. Therefore, further population-based association studies with large sample sizes are valuable to identify the real effect of the pre-miR-146a rs2910164 polymorphism on risk of gastric cancer.

| First author | Publication year | Country (region) | Ethnicity | Study design | Case/control | OR (95%CI)  | p-value
<table>
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<td>Asian</td>
<td>Case-control</td>
<td>268/324</td>
<td>1.16 (1.01-1.33)</td>
<td>0.025</td>
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<td>Xu 2012a</td>
<td>China (Chengdu)</td>
<td>Asian</td>
<td>Case-control</td>
<td>163/206</td>
<td>1.08 (0.92-1.26)</td>
<td>0.360</td>
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<td>Wang 2013</td>
<td>Japan (Toyoake)</td>
<td>Asian</td>
<td>Case-control</td>
<td>222/222</td>
<td>1.09 (0.95-1.25)</td>
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<td>Zou 2014</td>
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<td>Asian</td>
<td>Case-control</td>
<td>164/195</td>
<td>1.00 (0.86-1.16)</td>
<td>0.983</td>
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<tr>
<td>Stenholm 2015</td>
<td>Germany (Aachen)</td>
<td>European</td>
<td>Case only</td>
<td>603</td>
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<td>-</td>
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<td>Ma 2015</td>
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<td>Case-control</td>
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<td>Li 2016</td>
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<td>Case-control</td>
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<td>1.00 (0.86-1.16)</td>
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<td>Xu 2017</td>
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<td>Case-control</td>
<td>458/527</td>
<td>1.09 (0.95-1.26)</td>
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</table>

Association of the pre-miR-196a rs11614913 polymorphism with risk of gastric cancer

Overall, 13 studies reported the association between the pre-miR-196a rs11614913 polymorphism with risk of gastric cancer (Table 3) (Okubo et al., 2010; Peng et al., 2010; Ahn et al., 2013; Ma et al., 2013; Stenholm et al., 2013; Wang et al., 2013a; Dikeakos et al., 2014; Kupcinskas et al., 2014; Parlayan et al., 2014; Pu et al., 2014; Tábara et al., 2014; Xu et al., 2015). Among them, 8 were case-control studies, 3 were meta-analyses, and 2 were case-only studies. Similar to the pre-miR-146a rs2910164 polymorphism, most of the studies were conducted in Asian populations (Okubo et al., 2010; Peng et al., 2010; Ahn et al., 2013; Wang et al., 2013b; Parlayan et al., 2014; Pu et al., 2014; Tábara et al., 2014). The phenomenon of conflicting results is also observed with this polymorphism, even in the same population. Peng et al. (2010) reported that the pre-miR-196a rs11614913 CC genotype was associated with a significantly increased risk of gastric cancer in a Chinese population (OR = 1.57; 95%CI = 1.03-2.39). However, Pu et al. (2014) did not find any significant difference between cases and controls in another Chinese population. Similarly, meta-analysis was used to assess whether the pre-miR-196a rs11614913 polymorphism contributed to the risk of gastric cancer. No significant association was found between the pre-miR-196a rs11614913 polymorphism and gastric cancer risk by 3 independent groups (Ma et al., 2013; Wang et al., 2013a; Xu et al., 2015).
2015), suggesting that the pre-miR-196a2 rs11614913 polymorphism may not be a risk factor for the development of gastric cancer.

### Table 3. Association of the pre-miR-196a2 rs11614913 polymorphism with risk of gastric cancer.

<table>
<thead>
<tr>
<th>First author</th>
<th>Publication year</th>
<th>Country (region)</th>
<th>Ethnicity</th>
<th>Study design</th>
<th>Case/control</th>
<th>Case</th>
<th>Control</th>
<th>OR 95%CI</th>
<th>Association</th>
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</thead>
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<td>Wang</td>
<td>2013</td>
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<td>Ahn</td>
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<td>120</td>
<td>1.00</td>
<td>0.52-1.95</td>
</tr>
<tr>
<td>Zhang</td>
<td>2014</td>
<td>China (Shanghai)</td>
<td>Asian</td>
<td>Case-control</td>
<td>3035/3609</td>
<td>120</td>
<td>120</td>
<td>1.00</td>
<td>0.52-1.95</td>
</tr>
<tr>
<td>Ma</td>
<td>2014</td>
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<td>Case-control</td>
<td>3035/3609</td>
<td>120</td>
<td>120</td>
<td>1.00</td>
<td>0.52-1.95</td>
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</table>

Association of the pre-miR-196a2 rs229832 polymorphism with risk of gastric cancer

The association of the pre-miR-196a2 rs229832 polymorphism with risk of gastric cancer is presented in Table 4. In total, 6 studies were performed, including 4 case-control studies and 2 meta-analyses (Zhang et al., 2012; Ahn et al., 2013; Ma et al., 2013; Dikeakos et al., 2014; Pu et al., 2014; Xu et al., 2015). Zhang et al. (2012) reported that male subjects with the pre-miR-196a2 rs229832 CT genotype had a decreased susceptibility for gastric cancer in an Asian population (OR = 0.58; 95%CI = 0.37-0.93). Duplicate studies did not find the positive result in another two Asian populations (Ahn et al., 2013; Pu et al., 2014). The null result was also observed in meta-analyses (Ma et al., 2013; Xu et al., 2015). Nevertheless, Dikeakos et al. (2014) reported that the pre-miR-196a2 rs229832 polymorphism was associated with an increased risk of gastric cancer in a European population under a homozygote comparison (OR = 2.29; 95%CI = 1.25-4.22), dominant genetic model (OR = 1.47; 95%CI = 1.03-2.10), and recessive genetic model (OR = 2.00; 95%CI = 1.12-3.57). Taken together, these findings indicate that the pre-miR-196a2 rs229832 polymorphism may have different roles in diverse ethnicities with respect to gastric cancer risk.

### Table 4. Association of the pre-miR-196a2 rs229832 polymorphism with risk of gastric cancer.

<table>
<thead>
<tr>
<th>First author</th>
<th>Publication year</th>
<th>Country (region)</th>
<th>Ethnicity</th>
<th>Study design</th>
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</table>

Association of the pre-miR-196a2 rs3746444 polymorphism with risk of gastric cancer

The association of the pre-miR-196a2 rs3746444 polymorphism with risk of gastric cancer is summarized in Table 5. In total, 7 studies were performed, including 4 case-control studies, 2 meta-analyses, and 1 case-only study (Okubo et al., 2010; Ahn et al., 2013; Ma et al., 2013; Dikeakos et al., 2014; Pu et al., 2014; Xu et al., 2015). Zhang et al. (2012) reported that male subjects with the pre-miR-196a2 rs3746444 CT genotype had a decreased susceptibility for gastric cancer in an Asian population (OR = 0.58; 95%CI = 0.37-0.93). Duplicate studies did not find the positive result in another two Asian populations (Ahn et al., 2013; Pu et al., 2014). The null result was also observed in meta-analyses (Ma et al., 2013; Xu et al., 2015). Nevertheless, Dikeakos et al. (2014) reported that the pre-miR-196a2 rs3746444 polymorphism was associated with an increased risk of gastric cancer in a European population under a homozygote comparison (OR = 2.29; 95%CI = 1.25-4.22), dominant genetic model (OR = 1.47; 95%CI = 1.03-2.10), and recessive genetic model (OR = 2.00; 95%CI = 1.12-3.57). Taken together, these findings indicate that the pre-miR-196a2 rs3746444 polymorphism may have different roles in diverse ethnicities with respect to gastric cancer risk.

### Table 5. Association of the pre-miR-196a2 rs3746444 polymorphism with risk of gastric cancer.

<table>
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<th>Country (region)</th>
<th>Ethnicity</th>
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Association of the pre-miR-27a rs895819 polymorphism with risk of gastric cancer

It has been identified that miR-27a functions as an oncogene and is upregulated in gastric cancer, with higher expression resulting in significantly worse overall survival. Downregulation of miR-27a may inhibit proliferation of gastric cancer cells and drug resistance, indicating that miR-27a plays a key role in the carcinogenesis of gastric cancer. Sun et al. (2010) reported that a polymorphism (rs895819) within miR-27a might contribute to gastric cancer susceptibility by modulating the expression of miR-27a and its target gene zinc finger and BTB domain containing 10. Duplication studies in Asian populations found that the pre-miR-499 rs3746444 A allele was a predictive factor for better overall survival (HR = 0.33; 95%CI = 0.18-0.75), indicating that the pre-miR-499 rs3746444 polymorphism may be used as a biomarker for prognosis of advanced gastric cancer treated with chemotherapy (Tahara et al., 2014).

In this study, we reviewed miRNA-related polymorphisms in gastric cancer. In total, 45 studies reported the relationship of SNPs with gastric cancer risk. Among them, the most frequently studied polymorphisms were rs2910164, rs11614913, rs2292832, rs3746444, and rs895819, which are located in pre-miR-146a, pre-miR-196a2, pre-miR-149, pre-miR-499, and pre-miR-27a, respectively. Except for pre-miR-146a rs2910164 and pre-miR-27a rs895819, the polymorphisms were not associated with risk of gastric cancer. Therefore, functional
polymorphisms for epidemiological research are of great importance to identify susceptibility genes. Moreover, gene-gene and gene-environment analyses will provide more evidence for the better understanding of tumorigenesis in gastric cancer.

Conflicts of interest

The authors declare no conflict of interest.

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