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ORIGINAL RESEARCH

Single-nucleotide polymorphisms of microRNA processing machinery genes are associated with risk for gastric cancer

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Abstract: Recent studies demonstrate that microRNA-related single-nucleotide polymorphisms (miR-SNPs) are associated with the development of numerous human cancers. In this study, we investigated six miR-SNPs in microRNA processing machinery genes, including rs11077 of the *XPO5* gene, rs14035 of the *RAN* gene, rs3742330 of the *Dicer* gene, rs9623117 of the *TNRC6B* gene, rs197412 of the *GEMIN3* gene, and rs2740348 of the *GEMIN4* gene, in gastric cancer patients and subsequently evaluated their potential roles in gastric cancer risk in a case control study. The results indicate that the C/C genotype of rs14035 from *RAN*, the A/A genotype of rs3742330 from *Dicer*, and the T/T genotype of rs9623117 from *TNRC6B* are significantly associated with gastric cancer risk. In conclusion, these miR-SNPs can be used as predictive biomarkers in gastric cancer.

Keywords: cancer risk, gastric cancer, miR-SNPs

Introduction

Gastric cancer (GC) is the fourth-most-common cancer and the second leading cause of cancer-related deaths worldwide;[1,2] in addition, nearly two-thirds of GCs occur in developing regions.[3] As a multifactorial disease, both environmental factors and genetic factors contribute to the etiology of this disease.[4,5] Although much treatment progress has been achieved in recent years, the prognosis of GC remains poor due to the delay in diagnosis.[6] MicroRNAs (miRNAs) are RNA molecules with lengths of ~22 nucleotides; they can act as posttranscriptional regulators in mRNA expression, and they regulate a variety of physiological and developmental processes, including the development and progression of numerous cancers.[7–13] In GC, miRNAs can serve as biomarkers for both diagnosis and therapeutic targets.[14–17]

In miRNA processing, long primary transcripts of miRNAs (pre-miRNAs) are synthesized by RNase II in the nucleus, and they are cut into an ~70 nt hairpin precursor by RNase III Drosha and by the double-stranded RNA-binding protein DiGeorge syndrome critical region 8 (DGCR8). These precursors are exported to the cytoplasm, are processed by exportin-5 (*XPO5*) and RAN-GTPase (*RAN*), and are further diced into ~22 nt miRNA duplexes by the RNase III *Dicer* gene, which cooperates with the transactivation response RNA-binding protein and the Argonaute protein family. MiRNA duplexes are then assembled into the miRNA-induced silencing complex (miRISC), which contains *GEMIN3* and *GEMIN4*. The miRISC will select one strand as the mature miRNA, and it guides the mature miRNAs to their target mRNA sites.[18–23] MiRNA-related single nucleotide polymorphisms (miR-SNPs), which are defined as single-nucleotide polymorphisms (SNPs) in miRNA genes at the miRNA binding site

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and in miRNA processing machinery, can modulate miRNA and target gene expression to influence cancer development, promote therapeutic efficacy, and affect a patient's prognosis.[23–26] The miRNA machinery genes are linked to the development, progression, and prognosis of several cancer types, including ovarian and colorectal cancers, melanoma, and T-cell lymphoma.[27–30] However, the role of the miRNA processing machinery genes in GC remains uncertain.

In the present study, we genotyped six miR-SNPs in the miRNA processing machinery genes, including *XPO5* (rs11077), *RAN* (rs14035), *Dicer* (rs3742330), *TNRC6B* (rs9623117), *GEMIN3* (rs197412), and *GEMIN4* (rs2740348) in GC patients to evaluate the relationships of these genes with risk for development of GC.

Materials and methods

Sample collection and DNA extraction

Blood samples were collected from GC patients who underwent GC resection at the Department of General Surgery at the Fourth Hospital of Hebei Medical University from 2007–2008. Blood samples were also collected from normal controls without a history of any cancer. Genomic DNA was immediately extracted using the Wizard® Genomic DNA Purification Kit (Promega Corporation, Fitchburg, WI, USA) and was stored at –20°C. All procedures were supervised and approved by the Human Tissue Research Committee of the Fourth Hospital of Hebei Medical University. Written consent was obtained from all the patients and healthy controls enrolled in this study.

Genotyping of miR-SNPs

The miR-SNPs of the miRNA processing gene, including *XPO5* (rs11077), *RAN* (rs14035), *Dicer* (rs3742330), *TNRC6B* (rs9623117), *GEMIN3* (rs197412), and *GEMIN4* (rs2740348), according to the National Center for Biotechnology Information (NCBI) SNP database (http://www.ncbi.nlm.nih.gov/snp/), were genotyped using the polymerase chain reaction (PCR)–ligase detection reaction assay with the primers and probes listed in Table 1. PCR was performed with the PCR Master Mix Kit according to the manufacturer's instructions (Promega, Madison, WI, USA). Ligation was performed using the different probes that were matched to the miR-SNPs, and the ligated products were separated using the ABI PRISM Genetic Analyzer 3730XL (Applied Biosystems, Foster City, CA, USA) to access the length differences of the ligated products.

Table 1 All the primers and probes used for genotyping of miR-SNPs

Gene	rs NCBI	Primer sequence	Probe sequence
XPO5	rs11077 (A/C)	F GAATCTGGTCACCTGATGGGA	S1 GTACCTCCAAGGACCAGGGCTGGGA
		R GTGCCTGAGTGGACCTTGAG	S2 TTTGTACCTCCAAGGACCAGGGCTGGGC
			S3 AGTCTTTAGTGCTAACATCCCCTTT
RAN	rs14035 (C/T)	F GCACTTGCTCAAAATCTGTGA	S1 TTTTAGTAATCATGTTTTAATGTAGAACC
		R TAACAGCAAGAATTCCCAACCCT	S2 TTTTTTTAGTAATCATGTTTTAATGTAGAA
			S3 TCAAACAGGATGGAACATCAGTGGATTT
GEMIN4	rs2740348 (G/C)	F TTGCCTCTGAGAAGAAGTGG	S1 TTTTTTTTGGGAGTAACAGGGCCCTCTTCCGAC
		R GACTCAGGGATGGCTCTGTC	S2 TTTTTTTTTTGGGAGTAACAGGGCCCTCTTCCGAG
			S3 AGCCAGACTTGGTGTTGAGGCTGCTTTTTTT
TNRC6B	rs9623117 (C/T)	F TTTCT GTCTCCTCCTATCCAT	S1 TCTCCCTGTTACTCTTAAGTAGTGC
		R CATTAGTTTAGCCAACAAGGT	S2 TTTTCTCCCTGTTACTCTTAAGTAGTGT
			S3 CTCCTTTCCCCATCCACCCCATCTC
GEMIN3	rs197412 (T/C)	F TAGAGAAACCTGTGGAAATCA	S1 TTTTATGGTTTTGTGAGAAATAAAGTTAC
		R GAAGAGGTTCTTGAGCTGTAA	S2 TTTTTTTATGGTTTTGTGAGAAATAAAGTTAT
			S3 TGAACAGAGAGTCCCTGTGTTGGCATTT
Dicer	rs3742330 (A/G)	F AAAGGTATCAAGGTCTCAGTTTG	S1 TTTTTTTTTTCAATCTTGTGTAAAGGGATTAGA
		R CTGCAGAGGATCACTGGAATC	S2 TTTTTTTTTTTTTCAATCTTGTGTAAAGGGATTAGG
			S3 CACCCTAACAGAGCAAGATCCAATATTTTTT

Notes: F: represents forward primer for PCR; R: represents reverse primer for PCR; S1 and S2 represent probes match to different allele of the SNP; S3 represents probes downstream of the SNP.
Abbreviations: NCBI, National Center for Biotechnology Information; PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism.

Statistical analysis

The χ^2 test was applied to analyze dichotomous variables, such as the presence or absence of an individual SNP in patients and healthy controls. The odds ratios and 95% confidence intervals (CI) were calculated using an unconditional logistic regression model. All of the statistical analyses were performed with the SPSS version 18.0 software package (IBM Corporation, Armonk, NY, USA). A *P*-value <0.05 was considered statistically significant.

Results

A total of 137 GC patients and 142 healthy controls were included in this study. The clinical characteristics of the cases and controls are listed in Table 2. Six miR-SNPs were detected from the blood samples of the healthy controls and GC patients.

When individual SNPs were compared between GC patients and controls in terms of their distribution frequency, statistically significant increases for the C/C genotype of rs14035 in the *RAN* gene (95% CI: 1.601–14.925; *P*=0.002), the T/T genotype of rs9623117 in the *TNRC6B* gene (95% CI: 0.03–0.608; *P*=0.003), and the A/A genotype of rs3742330 in the *Dicer* gene (95% CI: 1.223–3.178; *P*=0.005) were observed in GC patients. These results indicate that the carriers of these alleles are susceptible to GC (Table 3).

Table 2 Clinical characteristics in patients with gastric cancer and controls

Characteristic	Case	Control	*P*-value
Sex			0.959
Male	99	103	
Female	38	39	
Age (years)			0.598
≤60	68	66	
>60	69	76	
Clinical stage			
I + II	47		
III + IV	90		
Size of tumor(diameter)			
≤5 cm	73		
>5 cm	64		
Degree of differentiation			
Highly + moderately	88		
Poorly + undifferentiated	49		
Intravascular tumor emboli			
No	100		
Yes	37		
Tumor location			
Upper third	49		
Middle third	43		
Lower third	45		
Lymph node metastasis			
N0	42		
N1	31		
N2	31		
N3	33		
Postoperative chemotherapy			
No	64		
Yes	73		

Discussion

The miR-SNPs in *RAN*, *Dicer*, and *TNRC6B* were associated with the carcinogenesis of GC in our analysis. To our knowledge, this is the first report to indicate that the SNP sites in the miRNA processing machinery genes have predictive value for determining the incidence of GC.

RAN is a member of the Ras superfamily of GTPases and is essential for the translocation of pre-miRNAs from the nucleus to the cytoplasm. *XPO5* binds to pre-miRNA and RAN-GTPase in the nucleus (via the *XPO5–RAN* GTP–pre-miRNA heteroternary complex) to mediate the nuclear export of pre-miRNA in a *RAN* GTP-dependent manner.[31] Disruption of pre-miRNA nucleocytoplasmic transport would impair the production of mature miRNAs in cancer cells. The fact that *RAN* is overexpressed in some cancer cell lines, including colon cancer, implies its role in tumor transformation; moreover, *RAN* was reported to suppress the activation of C-Jun-NH_2-kinase and inhibit the apoptosis induced by an anticancer drug.[32,33] The rs14035 located in *RAN* might alter *RAN* expression, so as to initiate carcinogenesis by modulating the production of mature miRNAs.

Dicer was also implicated in the oncogenic process of several cancers, but the data were controversial; downregulated *Dicer* expression has been identified in lung cancer, ovarian cancer, nasopharyngeal cancers, breast cancer, and esophageal cancer, whereas upregulated *Dicer* expression was found in lung adenocarcinoma, colorectal cancer, and primary cutaneous T-cell lymphomas.[27,34–40] The mechanism underlying how the rs3742330 SNP modified the GC risk remains unclear; the location of this SNP in the 3′-untranslated region of *Dicer* might potentially influence the stability and expression of the gene.

TNRC6B (or KIAA1093), localized on the mRNA-degrading cytoplasmic P bodies, is one of the three Argonaute-interacting protein paralogs in vertebrates. They assist in the formation of miRNA ribonucleoparticles or miRISCs, so as to mediate miRNA-guided mRNA cleavage.[16,41–44] The T/T genotype of rs9623117 in *TNRC6B* has been found to be associated with prostate cancer risk. Alterations in *TNRC6B* gene expression due to genetic variations

Table 3 miR-SNPs frequency difference between patients with gastric cancer and controls

Variants	Gene	Genotype	Case	Control	*P*-value	OR	95% CI
rs11077	*XPO5*	A/A	119	123	0.953	1.021	0.511–2.040
		A/C + C/C	18	19			
rs14035	*RAN*	C/C	86	35	0.002	4.888	1.601–14.925
		C/T + T/T	51	107			
rs3742330	*Dicer*	A/A	75	54	0.005	1.971	1.223–3.178
		A/G + G/G	62	88			
rs9623117	*TNRC6B*	T/T	135	128	0.003	0.135	0.03–0.608
		C/T + C/C	2	14			
rs197412	*GEMIN3*	T/T	65	60	0.383	1.234	0.769–1.979
		C/T + C/C	72	82			
rs2740348	*GEMIN4*	G/G	110	115	0.928	1.027	0.572–1.845
		C/G + C/C	27	29			

Abbreviations: CI, confidence interval; miR-SNP, microRNA-related single-nucleotide polymorphism; OR, odds ratio.

of rs9623117 might perturb the levels of miRNA species normally under its control, thus contributing to carcinogenesis.[45]

The frequency distribution of these six SNPs and the patients' clinical characteristics (including their sex, age, and tumor stage) do not appear to be associated, as determined by our analysis (data not shown). These six miR-SNPs were analyzed for their relationship with postoperative survival in 95 patients for whom 3-year follow-up data were available. It was found that rs2740348 showed a marginally statistically significant association with survival (P=0.06; our unpublished data). These findings should be validated with a larger sample size.

Although the results of this study require further validation among a larger GC cohort, as well as in laboratory-based functional studies, our data are encouraging because they demonstrate that miR-SNPs can be used to predict the risk for developing GC.

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Author contributions

All authors made substantial contributions to data generation and analysis, drafting or critical revision of the manuscript, and approval for the final version to be published.

Disclosure

The authors report no conflicts of interest in this work.

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