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ORIGINAL ARTICLE

Association of microRNA-933 variant with the susceptibility to gastric cancer

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Summary

Purpose: *Common single-nucleotide polymorphisms (SNPs) in microRNAs (miRs) have been shown to be associated with susceptibility to several types of human cancer. However, the association of miR-933 rs79402775 with gastric cancer (GC) has not been explored.*

Methods: *The association between rs79402775 in miR-933 and the risk of GC was explored in Chinese population based on MassARRAY technology. A total 374 GC patients and 999 cancer-free controls were enrolled in this study.*

Results: *Compared with the wild-type GG, GA genotype was associated with a significantly decreased risk of GC (OR=0.542, 95% CI=0.299-0.983, p=0.044) in female subjects. Moreover, the variant was also associated with the expression of alpha fetoprotein (AFP) and carcinoembryonic antigen (CEA).*

Conclusions: *miR-933(rs79402775) may contribute to decreased susceptibility to GC and this SNP could be developed as a biomarker for GC prognosis.*

Key words: *gastric cancer, miR-933, single nucleotide polymorphism*

Introduction

It is universally acknowledged that GC, the second leading cause of cancer death worldwide, is also the most frequent malignancy of the gastrointestinal tract in the Japanese and East Asian populations [1].

In addition to Helicobacter pylori infection, other factors including viral infection, genetic predisposition, environment and excessive alcohol consumption also contribute to oncogenesis and development of GC. In the past decades, the diagnostic methods and therapeutic options have seen important improvement, but the prognosis for GC patients remains poor, especially in more advanced stages. Numerous researches demonstrated that early identification and diagnosis of GC seem to reduce GC-related death. Therefore, it is of crucial significance to identify effective diagnostic and prognostic markers for GC.

miRs are a family of short (~22 nucleotides), non-coding, endogenous RNA molecules which regulate gene expression at 3'-untranslated region by base pairing with target mRNAs , lead-

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ing to mRNA cleavage or translational repression [2-4]. It was demonstrated that miRs have been predicted to regulate appropriately a third of human genes suggesting that miRs are crucial regulators of various biological pathways [5]. Numerous research suggested that miRs are involved in a broad range of biological processes, including cell differentiation, proliferation and apoptosis , stress resistance, and fat metabolism [6]. Several recent reports show that miRs are found to regulate the expressions of oncogenes or tumor suppressor genes, indicating their possible role in cancer susceptibility [7-9]. For example, it was reported that miR-106b, miR-93, and miR-25 were overexpressed in GC [10], whereas miR-143 and miR-145 were downregulated in GC [11].

SNPs, one common type of variation in the human genome, are pervasive in pri-miRs, pre-miRs or mature miRs, particularly in the seed regions. They could be involved in various biological processes by interfering the expression and / or maturation and target recognition of miRs [12]. Numerous studies have demonstrated that SNPs play roles in the development and prognosis of some cancers such as papillary thyroid carcinoma, hepatocellular carcinoma, breast cancer and GC. Therefore, SNPs in miRs may be regarded as effective biomarkers for diagnosis and/or prognosis of cancers.

A previous study suggested that miR-933 may contribute to genetic susceptibility to thyroid tumors [13]. However, the relationship between miR-933 and genetic predisposition to GC in any kind of ethnic group is unclear. Thus, the aim of this study was to investigate whether miR-933 SNP rs79402775 was associated with GC, by analyzing 374 Chinese GC patients and 999 cancer-free controls.

Methods

Study population

The studied population comprised 374 GC patients collected from Huashan Hospital (affiliated to Fudan University, Shanghai) and Huadong Hospital (affiliated to Fudan University, Shanghai), Renji Hospital and Tongren Hospital (affiliated to School of Medicine, Shanghai Jiaotong University, Shanghai), and 175 Hospital of PLA (affiliated to Xiamen University, Fujian). All GC were diagnosed histologically. Non-cancer subjects without a history of cancer were recruited from the CMC Institute of Health Sciences (Taizhou, China). For GC patients, clinical indexes such as serum levels of AFP, CEA, the size, site and tumor foci were also collected. This study was approved by the Human Research Review Committee of Huashan Hospital, Fudan University (Shanghai, China). All participating subjects provided written informed consent.

DNA extraction and genotyping

Genomic DNA of each subject was extracted from the peripheral blood using AxyPrep™ Blood Genomic DNA Miniprep Kit (Axygen Biosciences, Union City, USA). The Genotyping of rs79402775 was obtained by Sequenom MassARRAY technique. The amplification primers were designed by MassARRAY Assay Design Software and synthetized by Invitrogen Corporation Shanghai Representative Office. The data was analyzed by TYPER Analyzer software 4.0.

Statistics

IBM SPSS Statistics (version 20) and Excel 2010 were applied to conduct the data analysis. Binary logistic regression was used to estimate the association between the genotypes/allele types of miR SNPs and the susceptibility to GC with adjustment of age, gender, smoking status and drinking status. Odds ratios (ORs) and 95% confidence intervals (CI) were also calculated in order to estimate the relative risk. Pearson chi-square

Table 1. General characteristics in gastric cancer patients and controls

Characteristics	*Cases (n=374)*	*Controls (n=999)*	*p value*
	n (%) or mean ± SD	*n (%) or mean ± SD*	
Age (years)	62.13±12.089	59.59±11.621	<0.001
Gender			<0.001
Male	267 (72.8)	726 (72.7)	
Female	100 (27.2)	273 (27.3)	
Smoking status			<0.001
Never	273 (76.5)	528 (52.9)	
Ever	84 (23.5)	471 (47.1)	
Drinking status			<0.001
Never	313 (88.2)	736 (73.7)	
Ever	42 (11.8)	263 (26.3)	

test of independence was used to assess the differences of genotype frequencies among different groups and estimate the association between the SNPs and critical clinical indexes in GC patients. All statistical tests were two-sided and the probability levels <0.05 were used as a criterion of significance.

Results

The characteristics of the subjects

The general characteristics of the subjects are summarized in Table 1. A total of 374 GC patients and 999 controls were involved in this study. The age of GC patients was higher than those of cancer-free subjects. It was shown that gender, smoking status and drinking status were significantly different between the cases and the controls (p<0.001).

The *variant in miR-933 and the risk of GC*

The genotype distribution of miR-933 (rs794 02775) in GC patients and controls are demonstrated in Table 2. As for miR-933 (rs79402775), the frequency distributions of genotypes or alleles did not display statistically significant differences between the GC patients and the controls (p>0.05). Further gender analysis (Table 3) demonstrated the association in females. We found that female subjects with the miR-933 GA genotype had a decreased risk of GC as compared with the GG genotypes (OR=0.542, 95% CI=0.299-0.983, p=0.044). However, this association was not observed in males (Table 4).

Association between rs79402775 and demographic characteristic in GC patients

Besides the risk of GC, the association of rs79402775 with the clinical indexes of GC such as AFP, CEA, CA19.9, CA72.4 and the size, site and metastasis of tumor foci was also examined. As shown in Table 5, rs79402775 was significantly associated with serum level of AFP (p=0.044, 5) and CEA (p=0.015, Table 5). Compared to G allele of rs79402775, the A allele tended to have lower AFP and CEA level which indicated that the rs79402775 was significantly associated with the risk of GC. There was no significant association observed between the SNP and other clinical characteristic including CA19.9, CA72.4 and the size, site and metastasis of tumor foci.

Table 2. Association between genotypes/alleles of miR-933 rs79402775 and the risk of GC cancer

Genotypes	*Controls*	*Gastric cancer patients*		
	n (%)	*n (%)*	*OR (95% CI)*	*p value*
miR-rs79402775	n=999	n=374		
GG	736 (73.7)	278 (74.3)	1.000	
GA	245 (24.5)	85 (22.7)	0.852 (0.630-1.154)	0.302
AA	18 (1.8)	11 (2.9)	1.748 (0.787-3.881)	0.170
Dominant model (GG vs GA+AA)			0.912 (0.682-1.218)	0.532
Recessive model (GG+GAvs AA)			1.816 (0.820-4.020)	0.141
G	1717 (85.9)	641 (85.7)	1.000	
A	281 (14.1)	107 (14.3)	0.985 (0.762-1.273)	0.907

All ORs and p values were obtained after adjusting for age, gender, smoking status and drinking status

Table 3. Comparison of genotype/allele frequencies of miR-933 rs79402775 in female subjects

Genotypes	*Controls*	*Gastric cancer patients*		
	n (%)	*n (%)*	*OR (95% CI)*	*p value*
miR- rs79402775	n =273	n = 99		
GG	191 (70.0)	77 (77.0)	1.000	
GA	75 (27.5)	19 (19.0)	0.542 (0.299-0.983)	0.044
AA	7 (2.6)	4(4.0)	1.352 (0.380-4.812)	0.641
Dominant model (GG vs GA+AA)			0.612 (0.351-1.065)	0.082
Recessive model (GG+GA vs AA)			1.559 (0.442-5.501)	0.490
G	457 (83.7)	173 (86.5)	1.000	
A	89 (16.3)	27 (13.5)	0.728 (0.450-1.178)	0.196

ORs and p values were obtained after adjusting for age, gender, smoking status and drinking status

Table 4. Comparison of genotype/allele frequencies of miR-933 rs79402775 in male subjects

Genotypes	*Controls*	*Gastric cancer patients*		
	n (%)	*n (%)*	*OR (95% CI)*	*p value*
miR-646 rs6513497	n =726	n =264		
GG	545 (75.1)	196 (73.4)	1.000	
GA	170 (23.4)	64 (24.0)	1.015 (0.712-1.449)	0.933
AA	11 (1.5)	7 (2.6)	1.999 (0.714-5.594)	0.187
Dominant model (GG vs. GA+AA)			1.07 1(0.760-1.510)	0.693
Recessive model (GG+GA vs AA)			1.992 (0.714-5.554)	0.188
G	1260 (86.7)	456 (85.4)	1.000	
A	192 (13.2)	78 (14.6)	1.119 (0.825-1.519)	0.470

ORs and p values were obtained after the adjustment of age, gender, smoking status and drinking status

Table 5. Clinicopathologic characteristics and genotype/allele frequencies of miR-933 rs79402775 in GC patients

Characteristics	*Genotype*			*p value*	*Allele*		*p value*
	GG	*AG*	*AA*		*G*	*A*	
Tumor size (cm)	4.26±2.56	4.20±3.20	5.60±2.30	0.586	4.25±2.64	4.46±3.07	0.491
Tumor sites				0.976			0.886
Cardia cancer	174	50	6		398	62	
Non-cardia cancer	56	15	2		127	19	
Organ metastasis				0.524			0.274
Negative	192	55	6		439	67	
Positive	47	19	2		113	23	
Lymph-node metastasis				0.293			0.186
Negative	124	32	2		280	36	
Positive	135	40	7		310	54	
Differentiation				0.641			0.827
Poor	82	23	3		154	26	
Good and moderate	22	7	0		51	7	
AFP (ng/ml)				0.321			0.044
<10	32	46	11		146 (82.0)	32 (18.0)	
>10	24	57	12		168 (89.4)	20 (10.6)	
CEA (ng/ml)				0.893			0.015
<10	29	54	15		160 (81.6)	36 (18.4)	
>10	31	56	13		182 (90.1)	20 (9.9)	
CA19.9 (U/ml)				0.082			0.734
<37	20	26	9		96	14	
>37	10	36	10		96	16	
CA72.4 (U/ml)				0.115			0.492
<2	26	46	5		129	25	
>2	26	38	13		135	21	
CA50 (U/ml)				0.688			0.507
<25	10	18	7		59	13	
>25	10	25	6		71	11	

Discussion

GC is one of the common causes of cancer-related death worldwide, representing a major global health issue. Nearly 1 million cases of GC are diagnosed per year worldwide and account for >730,000 deaths [14]. Although the incidence of GC is declining, the mortality rate remains high in

the recent decade because of the lack of effective biomarkers to detect GC early and predict recurrence [15]. Current tumor markers for GC, including serum CEA and 19.9, have suboptimal sensitivity and specificity in the diagnosis and prognosis of GC [16-18]. Therefore, it is urgent to identify outstanding novel biomarkers for early detection of GC as well as for patient stratification towards individualized therapies.

Recently, numerous studies have revealed that many miRs are up- or down-regulated in GC, such as miR-25, miR-129, miR-199a-3p and miR-630 [19-22]. It was suggested that miRs could serve as a valid and noninvasive method for prediction and diagnosis of GC in addition to altered expression of oncogenes and tumor suppressor genes [23,24]. SNPs in miRs are able to influence the biogenesis and functions of their host miRs, thus contributing to susceptibility to cancers [25]. miRs could be released via secreting exosome particles, which could protect them from RNase degradation in the circulation [26]. With their stable expression of miRs in tissues and serum, they have emerged as novel cancer biomarkers [27].

In our study, the association between GC and the variant of miR-933 in a large-scale of Chinese population was investigated. After adjusting for age, sex, smoking and alcohol status, a significantly decreased risk of GC in females with the GA genotype was observed, compared with the wide-type homozygote GG. In the present study, the association between clinicopathological characteristics such as AFP, CEA and the variant of miR-933 was also observed which suggested that the variant of miR-933 may be used as an early detection indicator of GC.

In conclusion, we demonstrated that the miR-933 polymorphism is significantly associated with susceptibility to GC and rs79402775 SNP in miR-933 may have an effect on the clinicopathological characteristics of GC. To the best of our knowledge, this is the first study to explore the influence of miR-933 variant on the oncogenesis of GC. Recently, Wei et al. [13] identified that rs79402775 in miR-933 was associated with papillary thyroid cancer risk. All these findings indicated that rs79402775 has a crucial effect on the development and prognosis of cancer, which can serve as a biomarker for prediction and diagnosis of cancer. To better understand the relationship between rs79402775 and cancer risk , more functional studies of miR-933 and this SNP are suggested.

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Conflict of interests

The authors declare no conflict of interests.

References

1. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. Int J Cancer 1999;80:827-841.
2. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science 2001;294:853-858.
3. Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. Science 2001;294:858-862.
4. Lee RC, Ambros V. An extensive class of small RNAs in Caenorhabditis elegans. Science 2001;294:862-864.
5. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005;120:15-20.
6. Ambros V. MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing. Cell 2003;113:673-676.
7. Lu J, Getz G, Miska EA et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834-838.
8. Esquela-Kerscher A, Slack FJ. Oncomirs microRNAs with a role in cancer. Nat Rev Cancer 2006;6:259-269.
9. Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T. Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nat Genet 2007; 39: 673 - 677.
10. Takagi T, Iio A, Nakagawa Y, Naoe T, Tanigawa N, Akao Y. Decreased expression of microRNA-143 and -145 in human gastric cancers. Oncology 2009;77:12-21.

11. Petrocca F, Visone R, Onelli MR et al. E2F1-regulated microRNAs impair TGF beta-dependent cell-cycle arrest and apoptosis in gastric cancer. Cancer Cell 2008;13:272-286.
12. Slaby O, Bienertova-Vasku J, Svoboda M, Vyzula R. Genetic polymorphisms and microRNAs: new direction in molecular epidemiology of solid cancer. J Cell Mol Med 2012;16:8-21.
13. Wei WJ, Wang YL, Li DS et al. Association study of single nucleotide polymorphisms in mature microRNAs and the risk of thyroid tumor in a Chinese population. Endocrine 2015;49:436-444.
14. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA: Cancer J Clinicians 2012;62:10-29.
15. Kanda M, Kodera Y. Recent advances in the molecular diagnostics of gastric cancer. World J Gastroenterol 2015;21:9838-9852.
16. Duraes C, Almeida GM, Seruca R, Oliveira C, Carneiro F. Biomarkers for gastric cancer:prognostic,predictive or targets of therapy? Virchows Arch 2014;464:367 -378.
17. Emoto S, Ishigami H, Yamashita H, Yamaguchi H, Kaisaki S, Kitayama J. Clinical significance of CA125 and CA72-4 in gastric cancer with peritoneal dissemina-tion. Gastric Cancer 2012;15:154-161.
18. Kanda M, Fujii T, Takami H et al. Combina -tion of the serum carbohydrate antigen 19-9 and carcinoembryonic antigen is a simple and accurate predictor of mortality in pancreatic cancer patients. Surg Today 2014;44:1692-1701.
19. Li BS, Zuo QF, Zhao YL et al. MicroRNA-25 promo- tes gastric cancer migration, invasion and proliferation by directly targeting transducer of ERBB2, 1 and correlates with poor survival. Oncogene 2015; 34:2556-2665.
20. Gaur A, Jewell DA, Liang Y et al. Characterization of microRNA expression levels and their biological correlates in human cancer cell lines. Cancer Res 2007;67:2456-2468.
21. Li C, Li JF, Cai Q et al. miRNA-199a-3p in plasma as a potential diagnostic biomarker for gastric cancer. Ann Surg Oncol 2013; 3: 397 -405.
22. Chu D, Zhao Z et al. Increased microRNA-630 expression in gastric cancer is associated with poor overall survival. PLoS One 2014;9:e90526.
23. Adam J, Bass VT, Shmulevich I et al. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014;513: 202 - 209.
24. Janjigian YY, Kelsen DP. Genomic dysregulation in gastric tumors. J Surg Oncol 2013;107:237-242.
25. Ryan BM, Robles AI, Harris CC. Genetic variation in microRNA networks: the implications for cancer research. Nat Rev Cancer 2010;10:389-402.
26. Aravalli RN, Steer CJ, Cressman EN. Molecular mechanisms of hepatocellular carcinoma. Hepatology 2008;48:2047-2063.
27. Wu HH, Lin WC, Tsai KW. Advances in molecular biomarkers for gastric cancer: miRNAs as emerging novel cancer markers. Expert Rev Mol Med 2014; 16 :e1.