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A polymorphism at the miR-502 binding site in the 3′ untranslated region of the *SET8* gene is associated with the risk of epithelial ovarian cancer

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MicroRNAs (miRNAs) bind to the 3′ untranslated regions (UTRs) of messenger RNAs, where they interfere with the translation of genes that regulate cell differentiation, apoptosis, and tumorigenesis. *SET8* reportedly methylates *TP53* and regulates genomic stability. We analyzed a single nucleotide polymorphism (rs16917496) within the miR-502 mRNA seed region of the 3′ UTR of *SET8* in Chinese epithelial ovarian cancer (EOC) patients. The *SET8* CC genotype was associated with a decreased risk of EOC in this case-control study. The analysis of genetic polymorphisms in miRNA binding sites may help identify subgroups of populations that are at high risk for EOC.

Keywords MicroRNA, *SET8*, EOC, cancer risk

Ovarian cancer is the third most common cancer of the female genital tract and the leading cause of cancer death associated with gynecologic tumors. Epithelial ovarian carcinomas (EOCs) include several histologic types, such as serous-papillary, mucinous, endometrioid, undifferentiated, and the no clear-cell type, and account for nearly 90% of all ovarian cancers (1,2). Despite improved clinical detection methods and therapies, the prognosis of EOC patients is poor due to a high recurrence rate. The severity of EOC and the lack of effective treatment strategies are the major challenges facing cancer researchers (2,3). Several epidemiological factors have been identified as risk factors for EOC (4,5); however, the mechanism underlying the development of this cancer remains unknown. Cancer risk–associated single nucleotide polymorphisms (SNPs) have been examined extensively in EOC patients, but genetic factors associated with this disease have not yet been identified (2,6–8).

MicroRNAs (miRNAs) are RNA molecules that are approximately 22 nucleotides in length and act as post-transcriptional regulators of mRNA expression by forming base pairs with the 3′ untranslated region (UTR) of a target mRNA to repress translation (9,10). A growing body of evidence suggests that miRNAs have important roles in many biological processes, such as embryonic development, cellular differentiation, proliferation, apoptosis, cancer development, and insulin secretion (9,10). More than 700 miRNAs have been identified in humans, and these miRNAs are responsible for regulating the expression of at least 30% of protein-coding genes (11). Specifically, miRNAs target nucleotides 2–8 at the 5′ end, or "seed region," of the 3′ UTR of the target mRNA. Perfect complementarity between the miRNA and its target mRNA sequence reduces protein levels due to RNA silencing (12,13). Increasing evidence suggests that SNPs in the 3′ UTR region targeted by miRNAs can alter the expression of target genes and thereby affect cancer risk (14,15). miRNA-binding SNPs have been examined extensively in recent genotyping studies (16–19).

SET8, which is regulated by miR-502 via the binding site in the 3′ UTR of the *SET8* mRNA, encodes a histone H4 lysine 20 monomethyltransferase that is implicated in normal cell cycle progression (20–22). Previous studies have suggested that the SNP rs16917496, which is located within the miR-502 binding site in the *SET8* 3′ UTR, modulates *SET8* expression and contributes to cancer risk and the early development of cancer (16,23). In this study, we genotyped

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this SNP in EOC patients and normal controls to assess its association with cancer risk.

Materials and methods

DNA extraction

Blood samples were collected at the Fourth Hospital of Hebei University, Shijiazhuang, P.R. China, from 342 EOC patients who underwent tumor resections in the Department of Gynecology and Obstetrics between 2002 and 2010. Blood samples were also collected from 344 healthy female controls. Histopathological diagnoses and clinical staging were classified according to the criteria of the International Federation of Gynecology and Obstetrics. The genomic DNA was immediately extracted using the Wizard Genomic DNA extraction kit (Promega, Madison, WI). All procedures were supervised and approved by the Human Tissue Research Committee at the hospital.

Genotyping of rs16917496

The SNP rs16917496 was genotyped using the ligase detection reaction (LDR) method with the forward and reverse primers, 5′-CCTGGTCAGTGGTCAGCAAAT-3′ and 5′-CTG GGAAACACGCTCAAAATC-3′, respectively, to amplify the DNA fragments flanking rs16917496 in the *SET8* 3′UTR using the sequence in the NCBI database (http://www.ncbi.nlm.nih.gov/snp/). PCR was performed using a PCR Master Mix Kit according to the manufacturer's instructions (Promega, Madison, WI). The ligation was performed using the probes S1 (5′-TTGTGGTTTAGCTTTGTATTTAAAC-3′), S2 (5′-TTT TTGTGGTTTAGCTTTGTATTTAAAT-3′), and S3 (5′-AAGG AAATAAACTTGAAAATTATTT-3′), and the ligated products were separated using the ABI PRISM Genetic Analyzer 3730XL (Applied Biosystems, Foster City, CA). Polymorphisms were confirmed based on the 3 bp difference in length for different alleles of rs16917496.

Statistical analysis

Hardy-Weinberg equilibrium analysis was performed to compare the observed and expected genotype frequencies. The χ^2 test was used to analyze dichotomous values, such as the presence or absence of an individual SNP in EOC patients and healthy controls. The odds ratio (OR) and 95% confidence interval (CI) were calculated using an unconditional logistic regression model. All of the statistical analyses were performed using the SPSS 11.5 software package (SPSS, Inc., Chicago, IL). A *P*-value <0.05 was considered statistically significant.

Results

A total of 342 EOC patients with tumors ranging from stage I to IV and including 135 serous-papillary, 32 mucinous, 96 endometrioid, 67 undifferentiated, and 12 no clear-cell type tumors were enrolled in this study (Table 1). The control group consisted of 344 women without any history of hereditary or malignant disease, which was confirmed by physical and imaging examinations. All patients and controls were the same nationality (Han Chinese) and were recruited from Shijiazhuang and surrounding areas in North China.

Table 1 Clinical characteristics of EOC patients and healthy controls

	EOC patients (n = 342)	Controls (n = 344)
Age (years)		
≤40	40	38
41−50	83	86
51−60	133	122
>60	86	98
Tumor histology		
Serous	135	
Mucinous	32	
Endometrioid	96	
Undifferentiated	67	
No cell type	12	
Tumor stage		
I/II	97	
III/IV	245	

The rs16917496 SNP was genotyped in the 342 patients and 344 controls. The *SET8* CC, CT, and TT genotype frequencies in the control samples were 13.1, 38.4, and 48.5%, respectively, and the distribution was in Hardy-Weinberg equilibrium. The C allele was the minor allele, consistent with previous reports (16,23). The distribution frequencies of the rs16917496 polymorphism in the EOC patients and controls were compared using the χ^2 test. We demonstrated that the distributions of the CC type were significantly different in EOC patients and controls (Table 2). Compared with the C/T and C/T+T/T genotypes, the CC genotype was associated with a lower risk of EOC carcinogenesis ($P = 0.013$, OR: 0.511, 95% CI: 0.301−0.869 for C/C vs. C/T; $P = 0.028$, OR: 1.756, 95% CI: 1.062−2.902 for C/C vs. C/T+T/T). The distributions of genotype frequencies were similar among patients with five different histological tumor types (data not shown).

Discussion

The SNP in the miR-502 binding site of the *SET8* 3′ UTR was examined to evaluate its association with EOC cancer risk. We demonstrated that the CC genotype of rs16917496 was associated with a low risk of EOC cancer. This study is the first to report the potential role of a common SNP within the miRNA binding site of *SET8* in the etiology of EOC.

As a methyltransferase, *SET8* modulates p53 expression by methylating lysine 382 of histones that are associated with the p53 genomic sequence. This methylation event suppresses the transcriptional activation of p53 target genes.

Table 2 Correlation between rs16917496 SNP and EOC cancer risk

Genotype/ allele	Cases n (%)	Controls n (%)	*P*-value	OR	95% CI
C/C	27 (7.9)	45 (13.1)		1.000	
C/T	155 (45.3)	132 (38.4)	0.013	0.511	0.301−0.869
T/T	160 (46.8)	167 (48.5)	0.080	0.627	0.371−1.058
C/T+T/T	315 (92.1)	299 (86.9)	0.028	1.756	1.062−2.902

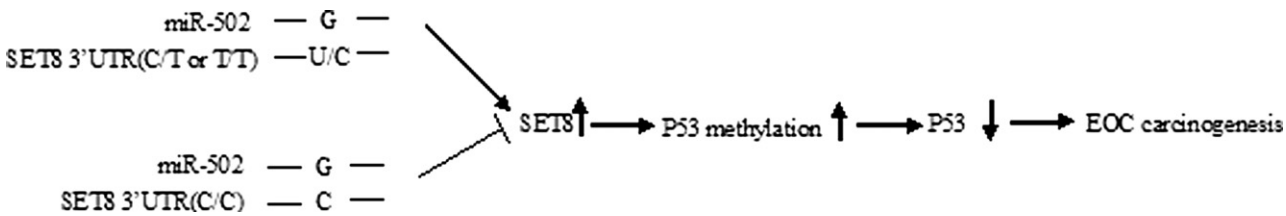

Figure 1 Proposed mechanistic relationship of the miR-502 binding site polymorphism in the *SET8* 3′UTR region with EOC carcinogenesis. The affinity for the C/T genotype of *SET8* versus miR-502 and the T/T genotype of *SET8* versus miR-502 is reduced in comparison to that of the C/C genotype of *SET8* versus miR-502 due to the mismatch of U in the *SET8* seed region. Thus, the presence of the T allele promotes p53 methylation by inducing higher *SET8* expression, and the resulting decrease in p53 level and change in the expression of its downstream genes initiates EOC carcinogenesis.

Furthermore, the depletion of *SET8* augments the pro-apoptotic and checkpoint activation functions of p53, and *SET8* expression is down-regulated by DNA damage (24). In a previous study, we found that *SET8* modifies hepatocellular carcinoma (HCC)outcome by altering *SET8* expression, which depends, at least in part, on its affinity to miR-502 (25). The proposed mechanism is depicted in Figure 1. We hypothesize that the affinity of miR-502 to the *SET8* 3′ UTR seed region is different for the C and T alleles. The C/T and T/T genotypes are associated with higher *SET8* expression, which alter p53 expression by affecting its methylation status, and the resulting decrease in p53 level initiates EOC carcinogenesis (Figure 1). *SET8* knockdown also enhances cell death and cell cycle arrest in response to DNA damage by suppressing the biological function of p53 (24).

Using case-control association studies, Yu et al. have found 12 miRNA binding site SNPs that display aberrant allelic frequencies in human cancers (16). The *SET8* miR-502 binding site SNP is one of these SNPs. We compared the distribution frequency of this SNP in a case-control study of EOC patients and confirmed the significant difference in the distribution frequencies of the *SET8* miR-502 binding-site SNPs in EOC patients and controls. Previously, this SNP was reportedly associated with early onset of breast cancer (23); however, no association was detected between EOC early onset and this SNP in the current study (data not shown). We also examined the predictive power of this SNP relative to post-surgery survival in EOC patients. No significant differences in EOC survival rates were detected among the three genotypes of this SNP (data not shown).

Although the studies of SNPs in miRNA binding sites are at an early stage, our results indicate that these variations have effects on cancer risk. However, the results of this study require validation in other populations and laboratory-based functional studies. MicroRNAs are key factors in patient therapeutic response in many complex diseases, including cancer (26). In conclusion, a SNP in the *SET8* microRNA binding site was identified as a marker for EOC cancer risk. The analysis of genetic polymorphisms in microRNA binding sites may help identify high-risk population subgroups and refine therapeutic decisions regarding EOC patients.

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References

1. Ahmed FY, Wiltshaw E, A'Hern RP, et al. Natural history and prognosis of untreated stage I epithelial ovarian carcinoma. J Clin Oncol 1996;14:2968–2975.
2. Li Y, Jia J-H, Kang S, et al. The functional polymorphisms on promoter region of matrix metalloproteinase-12, -13 genes may alter the risk of epithelial ovarian carcinoma in Chinese. Int J Gynecol Cancer 2009;19:129–133.
3. Fehrmann RS, Li XY, van der Zee AG, et al. Profiling studies in ovarian cancer: a review. Oncologist 2007;12:960–966.
4. Munksgaard PS, Blaakaer J. The association between endometriosis and gynecological cancers and breast cancer: a review of epidemiological data. Gynecol Oncol 2011;123:157–163.
5. Gram IT, Lukanova A, Brill I, et al. Cigarette smoking and risk of histological subtypes of epithelial ovarian cancer in the EPIC cohort study. Int J Cancer 2012;130:2204–2210.
6. Kang S, Wang D-J, Li W-S, et al. Association of p73 and MDM2 polymorphisms with the risk of epithelial ovarian cancer in Chinese women. Int J Gynecol Cancer 2009;19:572–577.
7. Li Y, Liang J, Kang S, et al. E-cadherin gene polymorphisms and haplotype associated with the occurrence of epithelial ovarian cancer in Chinese. Gynecologic Oncology 2008;108: 409–414.
8. Yan L, Na W, Shan K, et al. p16(CDKN2) gene polymorphism: association with histologic subtypes of epithelial ovarian cancer in China. Int J Gynecol Cancer 2007;18:30–35.
9. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–297.
10. Ambros V. The functions of animal microRNAs. Nature 2004; 431:350–355.
11. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicateds that thousands of human genes are microRNA targets. Cell 2005;120:15–20.
12. Zeng Y, Yi R, Cullen BR. MicroRNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms. Proc Natl Acad Sci U S A 2003;100:9779–9784.
13. Zeng Y, Wagner EJ, Cullen BR. Both natural and designed micro RNAs can inhibit the expression of cognate mRNAs when expressed in human cells. Mol Cell 2002;9:1327–1333.
14. Chin LJ, Ratner E, Leng S, et al. A SNP in a let-7 microRNA complementary site in the KRAS 3′ untranslated region increases non-small cell lung cancer risk. Cancer Res 2008;68: 8535–8540.
15. Brendle A, Lei H, Brandt A, et al. Polymorphisms in predicted microRNA-binding sites in integrin genes and breast cancer: ITGB4 as prognostic marker. Carcinogenesis 2008;29:1394–1399.
16. Yu Z, Li Z, Jolicoeur N, et al. Aberrant allele frequencies of the SNPs located in microRNA target sites are potentially associated with human cancers. Nucleic Acids Research 2007;35: 4535–4541.

17. Landi D, Gemignani F, Naccarati A, et al. Polymorphisms within micro-RNA-binding sites and risk of sporadic colorectal cancer. Carcinogenesis 2008;29:579–584.
18. Saunders MA, Liang H, Li W- H. Human polymorphism at microRNA and microRNA target sites. Proc Natl Acad Sci U S A 2007;9:3300–3305.
19. Gao Y, He Y, Ding J, et al. An insertion/deletion polymorphism at miRNA-122-binding site in the interleukin-1alpha 3′ untranslated region confers risk for hepatocellular carcinoma. Carcinogenesis 2009;30:2064–2069.
20. Fang J, Feng Q, Ketel CS, et al. Purification and functional characterization of SET8, a nucleosomal histone H4-lysine 20-specific methyltransferase. Curr Biol 2002;12:1086–1099.
21. Nishioka K, Rice JC, Sarma K, et al. PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin. Mol Cell 2002;9: 1201–1213.
22. Wu S, Wang W, Kong X, et al. Dynamic regulation of the PR-Set7 histone methyltransferase is required for normal cell cycle progression. Genes Dev 2010;24:2531–2542.
23. Song F, Zheng H, Liu B, et al. An miR-502-binding site single-nucleotide polymorphism in the 3′-untranslated region of the SET8 gene is associated with early age of breast cancer onset. Clin Cancer Res 2009;19:6292–6300.
24. Kachirskaia I Shi X, Yamaguchi H, et al. Modulation of p53 function by SET8-mediated methylation at lysine 382. Mol Cell 2007;27:636–646.
25. Guo Z, Wu C, Wang X, et al. A polymorphism at the miR-502 biding site in the 3′untranslated region of the histone methyl-transferase SET8 is associated with hepatocellular carcinoma outcome. Int J Cancer 2011 Nov 18 [Epub ahead of print].
26. Merritt WM, Lin YG, Han LY, et al. Dicer, Drosha, and outcomes in patients with ovarian cancer. N Engl J Med 2008;359: 2641–2650.