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New Phenomenon

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Association of SNPs in miR-146a, miR-196a2, and miR-499 with the risk of endometrial/ovarian cancer

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MicroRNAs (miRNAs) are a class of small non-coding RNA molecules (containing ~22 nucleotides), which function in RNA silencing and post-transcriptional regulation of gene expression [1]. miRNAs have been reported to play a key role in tumorigenesis [1]. Some single nucleotide polymorphisms (SNPs) in miRNA genes can influence the generation or function of miRNAs, and thereby possibly affect tumor growth and development.

The three SNPs we selected (miR-146a rs2910164, miR-196a2 rs11614913, and miR-499 rs3746444) had been found to have a close connection with the occurrence of multiple solid tumors [2–9]. However, the association between these SNPs and endometrial/ovarian cancer was not evaluated.

We performed a case–control study including 216 primary endometrial/ovarian cancer cases and 100 healthy controls. All subjects were Chinese Han population and were recruited from Obstetrics and Gynecology Hospital of Fudan University from June 2011 to February 2013. All of the people in both groups signed an informed consent and donated 5 ml peripheral blood for DNA extraction. Genomic DNA was extracted from peripheral blood using NaI and chloroform/isoamylol. Polymerase chain reaction (PCR)-restriction fragment length polymorphism was performed for genotyping. The PCR product was digested with restriction enzymes, followed by polyacrylamide gel electrophoresis on 8% gel, and stained with 0.1% $AgNO_3$. For quality control, the representative PCR products were randomly selected for sequencing validation, which yielded 100% consistent results. SPSS 18.0 and Epi info 7.0 were used for statistical analysis. To estimate the association between the risk of endometrial/ovarian cancer and SNPs, odds radio (OR) and 95% confidence interval (95% CI) were calculated using unconditional logistic regression model. And $P < 0.05$ was considered statistically significant.

As shown in Tables 1 and 2, the distribution of the three miR-146a rs2910164 genotypes was significantly different between endometrial/ovarian cancer cases and controls, with $P < 0.05$. For endometrial cancer, compared with GG genotype, CC and CG genotypes had lower frequencies (OR = 0.238, 95% CI = 0.110–0.516; OR = 0.143, 95% CI = 0.068–0.300) and individuals with CC/CG genotype are less susceptible to endometrial cancer (OR = 0.179, 95% CI = 0.090–0.356). For ovarian cancer, the frequencies of CC and CG genotypes were much lower than that of GG genotype (OR = 0.118, 95% CI = 0.048–0.294; OR = 0.120, 95% CI = 0.053–0.271). That is to say, compared with GG homozygote, CC and CG genotypes can significantly reduce the risk of endometrial cancer and ovarian cancer. Meanwhile, the C allele can be regarded as a protective factor for endometrial/ovarian cancer ($P < 0.05$). For mi196a2 rs11614913, there was no significant difference in genotypes distribution between endometrial cancer cases and controls ($P = 0.096$). However, the statistical data showed significant differences for rs11614913 genotype frequencies between ovarian cancer case and control groups ($\chi^2 = 7.305$, $P < 0.001$). The frequencies of CT genotype, TT and TT/CT genotypes were significant higher in cancer cases than in controls. Using CC genotype as reference, the OR values of TT, CT, and TT/CT genotypes were >1, which suggested that individuals carrying TT, CT, and CT/TT genotypes might have a 3.012 times, 3.677 times, and 3.435 times higher susceptibility to ovarian cancer, respectively (OR = 3.012, 95% CI = 1.046–8.675; OR = 3.677, 95% CI = 1.375–9.832; OR = 3.435, 95% CI = 1.321–8.930, respectively). Although the T allele distributed differently between cancer cases and controls, P value for T vs. C showed no difference. It ruled out the possibility that T allele is an independent risk factor for ovarian cancer. The distribution frequencies of the two genotypes of rs3746444

Table 1. Distribution of three SNPs in endometrial cancer and controls

Genotype	Cases ($n = 141$), n (%)	Controls ($n = 100$), n (%)	P	OR (95% CI)
miR-146a			<0.001[a]	
rs2910164 G > C				
GG	61 (43.3)	12 (12)		1.00 (reference)
CC	40 (28.4)	33 (33)		0.238 (0.110–0.516)
CG	40 (28.4)	55 (55)		0.143 (0.068–0.300)
CC + CG	80 (56.7)	88 (88)		0.179 (0.090–0.356)
C allele	120 (42.6)	121 (60.5)		
C vs. G			<0.001[b]	0.484 (0.334–0.699)
miR-196a2			0.096[a]	
rs11614913 C > T				
CC	19 (13.5)	23 (23)		1.00 (reference)
TT	36 (25.5)	28 (28)	0.267[a]	1.556 (0.711–3.405)
CT	86 (61.0)	49 (49)	0.033[a]	2.125 (1.053–4.285)
TT + CT	122 (86.5)	77 (77)	0.055[a]	1.918 (0.980–3.753)
T allele	158 (56.0)	105 (52.5)		
T vs. C			0.443[b]	1.153 (0.801–1.659)
miR-499			<0.05[a]	
rs3746444 T > C				
TT	123 (87.2)	77 (77)		1.00 (reference)
CC	0 (0)	0 (0)		–
CT	18 (12.8)	23 (23)	0.037[a]	0.494 (0.248–0.966)
CC + CT	18 (12.8)	23 (23)	0.037[a]	0.490 (0.248–0.966)
C allele	18 (6.4)	23 (11.5)		
C vs. T			0.047[b]	0.525 (0.275–1.001)

[a]P values for frequency distribution of genotypes.
[b]P values from χ^2 test.

Table 2. Distribution of three SNPs in ovarian cancer and controls

Genotype	Cases ($n = 141$), n (%)	Controls ($n = 100$), n (%)	P	OR (95%CI)
miR-146a			<0.001[a]	
rs2910164 G > C				
GG	40 (53.3)	12 (12)		1.00 (reference)
CC	13 (17.3)	33 (33)		0.118 (0.048–0.294)
CG	22 (29.3)	55 (55)		0.120 (0.053–0.271)
CC + CG	35 (46.6)	88 (88)		0.119 (0.056–0.254)
C allele	48 (32.0)	121 (60.5)		
C vs. G			<0.001[b]	0.313 (0.201–0.489)
miR-196a2			<0.001[a]	
rs11614913 C > T				
CC	6 (8.0)	23 (23)		1.00 (reference)
TT	22 (29.3)	28 (28)		3.012 (1.046–8.675)
CT	47 (62.7)	49 (49)		3.677 (1.375–9.832)
TT + CT	69 (92.0)	77 (77)		3.435 (1.321–8.930)
T allele	91 (60.7)	105 (52.5)		
T vs. C			0.128[b]	1.395 (0.908–2.144)
miR-499			0.959[a]	
rs3746444 T > C				
TT	58 (53.3)	77 (77)		1.00 (reference)
CC	0 (0)	0 (0)		–
CT	17 (22.7)	23 (23)		0.959 (0.481–2.003)
CC + CT	17 (22.7)	23 (23)		0.959 (0.481–2.003)
C allele	17 (11.3)	23 (11.5)		
C vs. T			0.961[b]	0.984 (0.505–1.915)

[a]P values for frequency distribution of genotypes.
[b]P values from χ^2 test.

showed marginal differences between endometrial cancer patients and controls ($\chi^2 = 4.340$, $P = 0.037$). The CT genotype of miR-499 rs3746444 showed marginal correlation with subdued risk of endometrial cancer when comparing with TT genotype (OR = 0.494, 95% CI = 0.248–0.966). In addition, a similar trend was observed in C allele compared with that in T allele (OR = 0.525, 95% CI = 0.275–1.001). While no statistically significant difference of rs3746444 genotype frequencies was found between ovarian cancer cases and control groups.

In conclusion, our results suggest that in Chinese population, miR-146a rs2910164 polymorphism can reduce not only the risk of endometrial cancer but also that of ovarian cancer, and miR-196a2 rs11614913 polymorphism can increase the risk of ovarian cancer. Meanwhile, miR-499 rs3746444 has potential function in reducing the risk of endometrial cancer. However, the small sample size of our study and the paucity of details of patient data might influence the statistical power. Further multicenter trials including the larger sample size are needed to verify our conclusions.

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