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RESEARCH ARTICLE

Association between *ERBB4* gene polymorphism in the microRNA binding site and endometrial carcinoma risk

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Abstract The relationship between the single-nucleotide polymorphism (SNP) of *ERBB4* gene in the microRNA binding site and the susceptibility of endometrial carcinoma was investigated. A total of 1671 patients with endometrial carcinoma and 1796 healthy controls were enrolled in the study. Genotypes of the *ERBB4* gene in the microRNA binding sites at rs1595066 and rs16845990 were then determined using the TaqMan SNP method. The endometrial carcinoma patients and healthy controls showed significant differences in three *ERBB4* gene genotypes in the microRNA binding site at rs1595066 ($P = 0.044$), whereas no difference was observed at the rs16845990 site ($P = 0.313$). Carriers of the AG and AA genotypes at the rs1595066 site showed lower risk of endometrial carcinoma than the carriers of the GG genotype [odds ratio (OR) = 0.82 and 95 % confidence interval (CI) = 0.70–0.94, and OR = 0.74 and 95 % CI = 0.56–0.94, respectively]. Stratified analysis showed that this protective effect was significant in subjects older than 50 years and those without a history of benign endometrial disease or a family history of cancer. The polymorphism of rs1595066 G > A in the microRNA binding site of the *ERBB4* gene is possibly associated with the reduction of endometrial carcinoma risk.

Keywords Endometrial carcinoma · MicroRNA · Single nucleotide and polymorphism

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Introduction

Endometrial carcinoma is among the leading causes of death in women worldwide and has become a major public health challenge (Jugurnauth et al. 2011). With the advancement of research on carcinogenesis, an increasing number of studies focus on novel strategies for early detection and prevention. The mechanisms of carcinogenesis are associated with low-penetrance susceptibility genes. However, this association has yet to be elucidated (Lichtenstein et al. 2000). The *ERBB4* gene, also known as the human epidermal growth factor receptor 4 gene, encodes the 4th member of the epidermal growth factor receptor family (Plowman et al. 1993) and consists of three parts, namely, the extracellular ligand-binding domain, transmembrane and intracellular areas. *ERBB4* participates in the regulation of cell growth, apoptosis and differentiation by mitogen-activated protein kinase, phosphatidylinositol 3-kinase and a series of cell signal-transduction pathways (Stenholm et al. 2013). In recent years, an increasing number of researchers (Metzeler et al. 2011; Yoon et al. 2012; Selcuklu et al. 2012; Smith et al. 2012) have focused on the involvement of the *ERBB4* gene in the formation and progression of endometrial carcinoma. MicroRNA (miRNA) molecules are vital in the regulation of approximately 30 % of protein-encoding genes, including multiple tumour-related genes, which strongly affect target gene expression and modify individual cancer susceptibility by binding to an mRNA target sequence at the 3′-untranslated region (Kumar et al. 2007).

The frequency of single-nucleotide polymorphisms (SNPs) is high because of the large number of target sequences and low conservation of untranslated regions (Saunders et al. 2007). Research on SNPs within the miRNA target sequences involves correlating changes in

the DNA sequence with gene expression. According to the data of the authority database "Patrocles" (http://www.patrocles.org/), which contains information on miRNA variations (Hiard et al. 2009), two SNPs (rs1595066 and rs16845990) are within the miRNA target sequences and are located in the miR-548 k and miR-185* binding sites in the 3′-untranslated region of the *ERBB4* gene. This study investigates the relationship between miRNA target-sequence SNPs in the 3′-untranslated region of the *ERBB4* gene and endometrial carcinoma susceptibility by analysing and comparing the peripheral blood of cancer patients and healthy volunteers.

Materials and methods

Subjects

A large case–control study was used. A total of 1671 cases of specimens were provided by The First, Second, and Third Affiliated Hospitals of Harbin Medical University. The patients were diagnosed with primary endometrial carcinoma based on pathology from December 2007 to August 2012. These patients had not received any previous treatment and had no history of other malignant tumours. The control group consisted of 1796 healthy female volunteers with negative results in endometrial carcinoma screening. The volunteers had no history of other malignant tumours, and their frequency of age and residence were matched to those of the patients. The control group was also subjected to an epidemiological investigation, including basic demographic information, fertility of menstruation information, history of disease or family history of cancer. This study was conducted in accordance with the declaration of Helsinki.

Specimen collection and genomic DNA preparation

Venous blood samples (5 mL) were collected from the patient and control groups in the early morning on an empty stomach. Genomic DNA was then extracted from the samples using the saturated salting-out method.

TaqMan SNP typing

TaqMan SNP typing was designed by ABI Company (North Carolina, N.C., USA), which was the same company that provided the primers and TaqMan MGB probe that counters rs16845990T > C and rs1595066 G > A SNP for real-time fluorescence PCR. PCR consisted of an initial degeneration step at 95 °C for 10 min, followed by amplification for 40 cycles at 92 °C for 15 s and then at 60 °C for 60 s. Sterilised water was used as the negative control. The consistency was 100 % after a 10 % random repeat.

Statistical analysis

Statistical analysis was performed using SAS version 9.0. The genotype conformity and the differences between genotype and allele frequency were determined using a two sided χ^2 test with $\alpha = 0.05$ as the standard. Odds ratios (ORs) and 95 % CI were estimated by single-factor and multifactor noncondition logistic regression analyses. A value of $P < 0.05$ was considered statistically significant.

Results

Environmental factors and endometrial carcinoma risk

In total, 1671 endometrial carcinoma patients and 1796 healthy controls were included in the study. The cases and controls were matched by age and other known information (Table 1). For both endometrial carcinoma patients and healthy controls, no statistically significant association was found for the oral contraceptives used, whereas a statistically significant association was found for menarche age, number of live births, family history and endometrial disease. Nonconditional logistic regression analysis showed that multiple factors, such as menarche age >12 years and the number of live births >2, were protective factors for endometrial carcinoma, whereas family history of tumours and history of benign endometrial disease increased the risk for endometrial carcinoma.

ERBB4 gene SNPs and endometrial carcinoma risk

General analysis showed no significant difference in the genotype distribution at rs16845990 of the case and control groups. Additionally, no statistically significant difference was found in the carrying C alleles of the two groups ($P = 0.142$) (Table 2).

General analysis also showed a statistically significant difference in the genotype distribution at rs1595066 of the case and control groups. Additionally, a statistically significant difference was found in the carrying A alleles of the two groups ($P = 0.013$). Compared with wild-type GG, heterozygous-type AG and homozygous-type AA can significantly reduce the risk for endometrial carcinoma. Moreover, carrying the A allele reduced the risk for endometrial carcinoma by 20 % after adjusting for factors such as age, history of benign endometrial disease and tumour family history (Table 2).

Table 1 General information of patients with endometrial carcinoma and healthy controls [n (%)]

Variable		Case (N = 1671)	Control (N = 1796)	OR (95 % CI)[a]	*P* value[b]
Age/year	≥50	1060 (63.43)	1121 (62.41)		0.549
	<50	611 (36.57)	675 (37.59)		
Menarche age/year[c]	>12	1506 (90.13)	1730 (96.33)	1	0
	≤12	165 (9.87)	66 (3.67)	1.91 (1.34–2.73)	
Live birth/n[c]	≤2	1458 (87.25)	1285 (71.55)	1	0
	>2	213 (12.75)	511 (28.45)	0.47 (0.37–0.59)	
Oral contraception	No	1363 (81.57)	1509 (84.02)	1	0.08
	Yes	308 (18.43)	287 (15.98)	1.18 (0.96–1.47)	
Benign endometrial disease[c]	No	1233 (73.79)	1680 (93.54)	1	0
	Yes	438 (26.21)	116 (6.46)	4.32 (3.36–5.54)	
Family history of cancer[c,d]	No	1158 (69.30)	1607 (89.48)	1	0
	Yes	513 (30.70)	189 (10.52)	3.19 (2.58–3.95)	

OR odds ratios, *CI* confidence interval

[a] Adjusted for age, menarche age, live birth number, menopause, oral contraception, benign endometrial disease, and family history of cancer

[b] Two-sided χ^2 test

[c] Data in the table are actual number of respondents

[d] First and second degrees of relatives

Table 2 Correlation of *ERBB4* gene SNPs in mirco-RNA binding site at rs16845990 and rs1595066 to endometrial carcinoma [n (%)]

Genotype	Case	Control	*P* value[a]	OR[b] (95 % CI)	*P* value
rs16845990[c]			0.313		
TT	513 (30.70)	507 (28.23)		1	
CT	810 (48.50)	914 (50.89)		1.01 (0.83–1.24)	0.895
CC	348 (20.80)	375 (20.88)		1.12 (0.90–1.39)	0.327
CC + CT	1158 (69.30)	1289 (71.76)		0.91 (0.76–1.07)	0.244
rs1595066			0.044		
GG	774 (46.32)	752 (41.87)		1	
AG	706 (42.25)	816 (45.43)		0.82 (0.70–0.94)	0.011
AA	191 (11.43)	228 (12.70)		0.74 (0.56–0.94)	0.023
AA + AG	897 (53.68)	1044 (58.13)		0.80 (0.68–0.93)	0.004

[a] Two-sided χ^2 test

[b] Adjusted for age, menarche age, live birth number, menopause, oral contraception, benign endometrial disease, and family history of cancer

[c] Data in the table are actual number of respondents

Stratified analysis

A stratified analysis of rs1595066 SNPs according to age, benign endometrial disease and family history of tumour showed that the A allele exhibited a protective effect. This protection was more significant in women younger than 50 years and without a family history of cancer or benign endometrial disease. No statistically significant variation was observed ($P = 0.104$, 0.442, 0.152) in the rs1595066 genotype with the age, history of benign endometrial disease and history of family tumour. A comprehensive analysis of the aforementioned factors showed that endometrial carcinoma risk decreased by 59 % in women carrying the AA genotype, younger than 50 years and had no history of benign endometrial disease or family history of cancer (Table 3).

Discussion

The human *ERBB4* gene is located on chromosome 2q34. A number of studies (Tang et al. 1999; Junttila et al. 2005; Zhu et al. 2006; Maatta et al. 2006; Hollmen et al. 2009) showed that *ERBB4* promotes the proliferation

Table 3 Stratified analysis for the genotype frequency of *ERBB4* gene in mirco-RNA binding site at rs1595066 and endometrial carcinoma risk

Variable		Case			Control			*P* value[a]	OR (95 % CI)[b]		
		GG	AG	AA	GG	AG	AA		GG	AG	AA
Age/year	≥50	477	450	133	464	520	137	0.216	1	0.83 (0.67–1.02)	0.92 (0.68–1.26)
	<50	297	256	58	288	296	91	0.038	1	0.78 (0.59–1.02)	0.51 (0.33–0.77)
Benign endom- etrial disease	No	582	508	143	699	769	212	0.017	1	0.77 (0.64–0.91)	0.74 (0.57–0.97)
	Yes	192	198	48	53	47	16	0.518	1	1.19 (0.73–1.92)	0.79 (0.39–1.58)
Family history of cancer	No	557	479	122	669	738	200	0.007	1	0.75 (0.63–0.90)	0.71 (0.54–0.94)
	Yes	217	227	69	83	78	28	0.725	1	1.12 (0.75–1.67)	0.93 (0.53–1.62)
Overall factor	Nagative	175	137	22	249	252	77	0.003	1	0.77 (0.57–1.04)	0.41 (0.24–0.70)
	Positive	599	569	169	503	564	151	0.191	1	0.85 (0.71–1.01)	0.93 (0.71–1.13)

[a] Two-sided χ^2 test

[b] Adjusted for age, menarche age, live birth number, oral contraception, benign endometrial disease, and family history of cancer; Negativity indicated that age <50 years, without benign endometrial disease and family history of cancer; Positivity indicated that age ≥50 years, with benign endometrial disease and family history of cancer

of endometrial carcinoma. Some scholars (Muraoka-cook et al. 2006; Lerman et al. 2010; Xing et al. 2012) considered *ERBB4* as a tumour suppressor gene. In in vitro experiments, Sartor (Sartor et al. 2001) found that endogenous and exogenous *ERBB4* exhibits an anti-proliferation effect on endometrial cancer cell after activation of neuronal ligand differentiation factors.

In this study, no significant relationship was found between the miR-185* target sequence SNP at rs16845990 in the *ERBB4* gene and the risk for endometrial carcinoma. A mutation of the A allele was found in the miR-548 k target sequences at rs1595066 in the *ERBB4* gene. According to the prediction of the miRNA mutation-related authority database "Patrocles" (http://www.patrocles.org/) (Rokavec et al. 2007), rs1595066 is a "transformational" target sequence SNP in which G alleles combine with miR-548 k and A alleles pair with miR-200a*. MiR-200a* belongs to the miR-200 family. Our study found that GG and heterozygous and homozygous AG AA at rs1595066 can reduce the endometrial carcinoma risk compared with their wild-type counterparts. A 20 % reduction in endometrial carcinoma risk was found in the presence of at least one A allele (AG/AA). This effect may be due to the downregulated *ERBB4* expression and reduced incidence of endometrial carcinoma after miR-200a* binds to the A allele.

Further analysis according to the age, history of benign endometrial disease or family history of tumour showed that the protection of rs1595066 G > A was more significant in women younger than 50 years and had no family history of cancer or benign endometrial disease. The history of benign endometrial disease and family history of tumour are strong risk factors that can negate the protective effect of rs1595066 SNP. No statistically significant difference was found between the age, history of benign endometrial disease, family history of tumour and rs1595066 genotypes. This finding is consistent with the aforementioned results. By considering the age, history of benign endometrial disease and family history of tumour and benign endometrial disease, the risk for endometrial carcinoma was low in women that carry the AA genotype, were younger than 50 years, and had no family history of cancer or benign endometrial disease.

In summary, the A allele in the miRNA target sequence G > A of the *ERBB4* gene at rs1595066 possibly significantly reduces the risk for endometrial carcinoma. The results of this study should be validated in Chinese populations and in other parts of the world. However, whether the combination of rs1595066 G > A with miRNA is consistent with the prediction in the "Patrocles" database remains to be confirmed, and the mechanisms underlying the reduction in the risk for endometrial carcinoma require further investigation.

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Compliance with ethical standards

Bioethical comments This study was conducted with approval from the Ethics Committee of the 2nd Affiliated Hospital of Harbin Medical University. Written informed consent was obtained from all participants.

Conflict of Interest We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or

company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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