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Original Article

Association between miR-125a rs12976445 and survival in breast cancer patients

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Abstract: MicroRNAs (miRNAs) act as an oncogene or a tumor suppressor by negatively regulating target genes. Genetic variants in miRNA genes confer susceptibility to cancer and risk of death in cancer patients. The aim of this study was to investigate whether miRNA polymorphisms were associated with survival in breast cancer patients. Five miRNA polymorphisms (miR-26a1 rs7372209, miR-125a rs12976445, miR-218 rs11134527, miR-423 rs6505162, and miR-608 rs4919510) were genotyped in 196 breast cancer patients. We found that miR-125a rs12976445 was significantly associated with survival in codominant, recessive, and dominant models. However, only association under the codominant model remained significant after adjustment for lymph node metastasis, TNM stage, estrogen receptor, and progesterone receptor. Furthermore, this effect remained in stratification analysis. In conclusion, our results provide evidence that miR-125a rs12976445 may serve as a prognostic biomarker for breast cancer. Further large-scale studies are required to confirm these findings.

Keywords: MicroRNA, miR-125a, polymorphism, breast cancer, prognosis

Introduction

Breast cancer remains the most frequently diagnosed cancer and the leading cause of cancer-related death worldwide in women [1], despite the improvement in adjuvant treatment and screening. Although there are varieties of treatment options for breast cancer, the choice of therapy in the metastatic setting remains limited. Some biomarkers, such as estrogen receptor (ER), progesterone receptor (PR), have been widely used to help guide treatment of breast cancer. However, many patients suffer a cancer relapse and eventually die of metastatic disease. Therefore, it is important to identify reliable prognostic biomarkers that can distinguish patients with a favorable or poor prognosis.

MicroRNAs (miRNAs) are a class of endogenous small non-coding RNA found in animals, plants, and other diverse eukaryotes as well as a number of DNA viruses. miRNAs negatively regulate gene expression at the post-transcriptional level by binding to the 3'-UTR of their target genes. To date, there are 2588 human mature microRNAs in miRBase 21 database [2]. Since a single miRNA can target hundreds of genes, miRNAs participate in the regulation of almost every cellular process. Aberrant expression of miRNA is involved in numerous human disease, including cancer [3-5]. Many studied have demonstrated that dysregulation of miRNAs not only affects carcinogenesis, but also promotes cancer progression and metastasis [3, 6-8]. miRNAs have great potential to serve as biomarkers for cancer detection, diagnosis and prognosis [6, 7, 9-11].

Previous studied have revealed that single nucleotide polymorphisms (SNPs) in miRNA genes affect mature miRNA expression and dis-

Table 1. Clinical characteristics of breast cancer patients

Characteristic	Number	%
Age (year)	53.3 ± 12.7	
Family history of cancer		
Yes	59	30.1
No	124	63.3
Known	13	6.6
Menopausal		
Premenopausal	104	53.1
Postmenopausal	92	46.9
LNM		
Negative	79	40.3
Positive	113	57.7
Unknown	4	2.0
TNM		
I	22	11.2
II	112	57.1
III	56	28.6
Unknown	6	3.1
ER status		
Positive	115	58.7
Negative	61	31.1
Unknown	20	10.2
PR status		
Positive	94	48.0
Negative	83	42.3
Unknown	19	9.7

rupt the ability of miRNAs to target genes [12-15]. miRNA SNPs are not only responsible for susceptibility to cancer, but also influence cancer prognosis [9, 16-18]. In the present study, we examined the relationship between five miRNA SNPs (miR-26a1 rs7372209, miR-125a rs12976445, miR-218 rs11134527, miR-423 rs6505162, and miR-608 rs4919510) and survival in breast cancer patients in a Chinese Han population.

Materials and methods

Patients

We retrospectively collected data of breast cancer patients after surgical resection of the primary tumor between 2001 and 2003. A total of 196 breast cancer patients were included in the final analysis after excluding patients with a history of cancer, other than breast cancer, or without survival status or clinical information. All specimens were archived in the Biobank of National Engineering Center for Biochip at Shanghai. The written informed consent was obtained from all patients before surgery, and the study protocol was approved by the Ethics Committees of Taizhou People's Hospital and National Engineering Center for Biochip at Shanghai.

Genotyping

Genomic DNA was extracted from five sections (5 μm each) of each FFPE tissue specimen using QIAamp DNA FFPE Tissue Kit (Qiagen, German) according to the manufacturer's protocol. The concentration and quality of genomic DNA was measured by NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, DE, USA) and then stored at -80°C until use. All SNPs were genotyped using polymerase chain reaction-ligation detection reaction (PCR-LDR) method as described previously [19].

Statistical analyses

Genotype frequencies of all SNPs were test for Hardy-Weinberg equilibrium using a goodness-of-fit Chi square test with 1 df. Codominant, recessive and dominant models were used for each SNP. For the codominant model, the common homozygote was used as reference group. The primary endpoint was overall survival (OS), which was calculated from the day of surgery to the date of the last follow-up or date of death due to any cause. OS estimates were calculated by using the Kaplan-Meier method, and the differences in OS between genotypes were compared by using log-rank tests. Hazard ratio (HR) and their 95% confidence intervals (CIs) of the effect for each SNP on the OS of breast cancer were calculated by using Multivariate Cox-proportional hazard model and were adjusted for lymph node metastasis (LNM), TNM stage, estrogen receptor status, and progesterone receptor status. All analyses were performed using SPSS software package (SPSS Inc., Chicago, IL, USA). All analyses were two-sided, and a P value < 0.05 was considered as statistically significant.

Results

Patient characteristics

The clinical and pathologic characteristics of 196 breast cancer patients included in the analyses were listed in **Table 1**. The median age at diagnosis was 51 years (range, 29-83

Table 2. Univariate and multivariate Cox regression analysis of overall survival in breast cancer patients

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (years), ≤ 50 *vs* > 50	0.612 (0.355-1.054)	0.077		
Family history of cancer, Yes *vs* No	0.650 (0.399-1.247)	0.195		
Menopausal, Premenopausal *vs* Postmenopausal	1.404 (0.819-2.409)	0.217		
LNM, Positive *vs* Negative	2.200 (1.215-3.984)	0.009	1.127 (0.494-2.568)	0.776
TNM, III *vs* I+II	2.609 (1.521-4.475)	< 0.001	1.661 (1.132-2.436)	0.009
ER, Positive *vs* Negative	0.379 (0.216-0.666)	0.001	0.519 (0.238-1.129)	0.098
PR, Positive *vs* Negative	0.446 (0.249-0.798)	0.007	0.685 (0.308-1.525)	0.355
rs11134527				
AA	1			
AG	1.456 (0.658-3.220)	0.354		
GG	1.187 (0.660-2.135)	0.567		
Dominant model	1.321 (0.646-2.701)	0.445		
Recessive model	1.245 (0.714-2.170)	0.441		
rs12976445				
CC	1		1	
CT	1.526 (0.876-2.658)	0.135	1.618 (0.884-2.960)	0.119
TT	4.024 (1.544-10.483)	0.004	3.964 (1.070-14.686)	0.039
Dominant model	1.709 (1.006-2.901)	0.047	1.702 (0.953-3.041)	0.072
Recessive model	3.357 (1.338-8.422)	0.010	2.134 (0.631-7.224)	0.223
rs6505162				
CC	1			
AC	0.636 (0.154-2.636)	0.533		
AA	0.660 (0.358-1.215)	0.182		
Dominant model	0.657 (0.367-1.175)	0.157		
Recessive model	0.723 (0.176-2.968)	0.653		
rs4919510				
CC	1			
GG	1.424 (0.652-3.112)	0.375		
CG	1.318 (0.721-2.408)	0.370		
Dominant model	1.344 (0.759-2.382)	0.311		
Recessive model	1.210 (0.610-2.402)	0.585		

years). Among these patients, 115 (58.7%) were ER-positive, 94 (48.0%) were PR-positive. The duration of follow-up ranged from 2.0 to 160.0 months with a median follow-up of 114.0 months. During the follow-up period, 55 (28.1%) patients were died. LNM, TNM stage, ER status, and PR status were significantly associated with survival time of patients (log-rank $P < 0.05$).

Association between miRNA SNPs and OS

Genotype frequencies of miR-26a1 rs7372209 deviated from Hardy Weinberg equilibrium ($P = 0.001$), and thus miR-26a1 rs7372209 was excluded from further analysis. The associations of miRNA SNPs with OS of breast cancer patients were presented in **Table 2**. In the univariate analysis of various clinical features for OS, LNM (HR = 2.200, 95% CI 1.215-3.984, $P = 0.009$), TNM stage III (HR = 2.609, 95% CI 1.521-4.475, $P < 0.001$), positive ER (HR = 0.379, 95% CI 0.216-0.666, $P = 0.001$), positive PR (HR = 0.446, 95% CI 0.249-0.798, $P = 0.007$), and miR-125a rs12976445 T allele (TT vs CC, HR = 4.024, 95% CI 1.544-10.483, $P = 0.004$; TT+CT vs CC, HR = 1.709, 95% CI 1.006-2.901, $P = 0.047$; TT vs CC+CT, HR = 3.357, 95% CI 1.338-8.422, $P = 0.010$) were associated with worse survival (**Figure 1**). In the multivariate analysis, only TNM stage (HR = 1.661, 95% CI 1.132-2.436, $P = 0.009$) and miR-125a

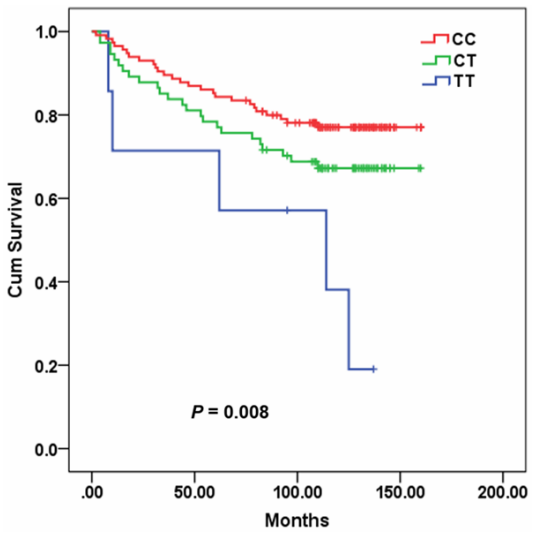

Figure 1. Plot of overall survival curve in breast cancer patients according to rs12976445 genotype.

rs12976445 TT genotype (HR = 3.964, 95% CI 1.070-14.686, *P* = 0.039) were independent unfavorable prognostic factors for breast cancer patients.

Stratified analysis

To further evaluate the effect of miR-125a rs12976445 on OS of breast cancer patient, stratification analyses were performed according to age, family history of cancer, menopausal, LNM, TNM stage, PR, and ER. In the different subgroups of patients, there was significant relationship between miR-125a rs12976445 and OS of breast cancer patients under codominant, recessive, or dominant models (**Table 3**).

Discussion

In the present study, we investigated the association between miRNA SNPs and OS in breast cancer patients. Our findings revealed that miR-125a rs12976445 TT genotype was significantly related to increased risk of mortality in breast cancer patients compared with those carrying the CC genotypes. Furthermore, multivariate Cox multivariate analysis demonstrates that miR-125a rs12976445 was an independent risk factor for poor survival in breast cancer.

miR-125a, located at chromosome 19q13.41, plays important roles both in organ development and in the adult tissues [5, 20, 21]. Growing evidence has demonstrated that miR-125a is implicated in the pathogenesis of human cancer, including breast, gastric and lung cancers [4, 22-24]. miR-125a functions as an oncogene or a tumor suppressor depending on the cellular context. For example, miR-125a was found downregulated in some types of cancer, including breast cancer [4], gastric cancer [22, 25], leukemia [5, 26], lung cancer [23, 24], and medulloblastoma [27], where its overexpression inhibited cancer cell proliferation and induced apoptosis. Furthermore, the effect of miR-125a on growth, migration and apoptosis of multiple myeloma cells is p53-dependant [21]. On the other hand, Kim et al. found that miR-125a was upregulated in diffuse large B-cell lymphoma, leading to enhanced NF-κB activity by targeting TNFAIP3 and thus promoting the development of a malignant and anti-apoptotic phenotype in B cells [28]. The expression level of miR-125a is not only associated with prognosis of cancer patients [22, 23], but also affects the sensitivity of cancer cell lines to anticancer drugs [26, 29].

Previous study revealed that germline mutation of miR-125a is associated with breast cancer tumorigenesis [30]. There are two SNPs (rs41275794 and rs12976445) in the pre-miR-125a gene in Chinese Han population [14], which are in complete linkage disequilibrium with each other. Rs12976445 contributes to the susceptibility to autoimmune thyroid diseases [31] and recurrent pregnancy loss [14]. Moreover, rs12976445 is associated with poor prognosis in patients with esophageal squamous cell carcinoma [9]. In this study, we found that patients carrying rs12976445 TT genotype had poor prognosis. Recent studies demonstrated that the T allele of rs12976445 impairs mature miRNA processing and expression of mature miR-125a, leading to increased expression of target genes, such as ERBB2 and LIFR [14, 15]. Amplification/overexpression of ERBB2 is related to a more aggressive behavior and a poor prognosis in breast cancer [32, 33], which is predictive biomarkers of trastuzumab. In addition, higher level of miR-125a implied better effect of gefitinib on nasopharyngeal carcinoma cells [29]. Accordingly, miR-125a rs12976445 may influence the effectiveness of trastuzumab.

In conclusion, our findings suggest that miR-125a rs12976445 are possible prognostic biomarkers for breast cancer patients. Further larger-scale and well-designed clinical studies are warranted to validate these findings.

Table 3. Stratification analysis of rs12976445 genotype associated with survival of breast cancer patients

Characteristics	Codominant model		Dominant model		Recessive model	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (years)						
≤ 50	4.212 (0.932-19.048)	0.062	1.350 (0.573-3.179)	0.492	3.975 (0.924-17.108)	0.064
> 50	3.854 (1.113-13.346)	0.033	2.043 (1.037-4.022)	0.039	2.919 (0.890-9.571)	0.077
Family history of cancer						
Yes	2.283 (0.281-18.565)	0.440	1.036 (0.328-3.268)	0.952	2.364 (0.305-18.321)	0.410
No	4.925 (1.133-21.406)	0.033	1.995 (1.044-3.810)	0.036	3.674 (0.882-15.303)	0.074
Menopausal						
Premenopausal	3.566 (0.818-15.555)	0.091	1.389 (0.702-2.749)	0.346	3.204 (0.766-13.411)	0.111
Postmenopausal	4.773 (1.309-17.404)	0.018	2.170 (0.937-5.023)	0.071	3.915 (1.157-13.251)	0.028
LNM						
Positive	3.502 (1.189-10.318)	0.023	1.718 (0.923-3.197)	0.088	2.936 (1.044-8.259)	0.041
Negative	4.602 (0.560-37.789)	0.155	1.782 (0.646-4.915)	0.264	3.675 (0.478-28.255)	0.211
TNM						
I+II	5.941 (1.342-26.303)	0.019	1.447 (0.690-3.035)	0.328	5.588 (1.316-23.727)	0.020
III	2.343 (0.652-8.415)	0.192	2.505 (1.133-5.539)	0.023	1.676 (0.501-5.613)	0.402
ER						
Positive	3.223 (0.706-14.719)	0.131	2.095 (0.919-4.779)	0.079	2.413 (0.566-10.294)	0.234
Negative	2.868 (0.367-22.430)	0.315	1.045 (0.483-2.260)	0.911	3.003 (0.399-22.578)	0.285
PR						
Positive	4.449 (0.897-22.065)	0.068	3.197 (1.199-8.525)	0.020	2.583 (0.594-11.237)	0.206
Negative	3.558 (0.462-27.379)	0.223	1.014 (0.497-2.068)	0.970	3.580 (0.480-26.684)	0.213

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Disclosure of conflict of interest

None.

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