RESEARCH ARTICLE

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The Association of *pre-miR27a* Gene Polymorphism and Clinicopathological Data in Thai Breast Cancer Patients

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Abstract

Background: MiR27a plays an important role in carcinogenesis, cell proliferation, apoptosis, invasion, migration and angiogenesis. Several studies have identified an important role of *pre-miR27a* (rs895819) A>G polymorphism in several types of cancer. This research aims to investigate the association of *pre-miR27a* (rs895819) A>G and breast cancer susceptibility, clinicopathological data and survival. Blood DNA samples of 143 Thai breast cancer patients and 100 healthy Thai women were studied for *pre-miR27a* (rs895819) A>G polymorphism using polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP). **Results:** The results revealed that the frequency of *pre-miR27a* (rs895819) A>G genotypes was not statistically significant different between breast cancer patient and normal control subjects. The rs895819 A>G genotype was significantly associated with clinicopathological parameter of grade III differentiation (P = 0.006), progesterone receptor (P = 0.011) and triple negative (P = 0.031) in breast cancer patients, but not with breast cancer susceptibility. **Conclusion:** The *pre-miR27a* (rs895819) A>G genotype was significantly associated with poorly differentiated, progesterone receptor and triple-negative in breast cancer patients. Therefore, *pre-miR27a* (rs895819) A>G may be used as a biomarker for poor prognosis.

Keywords: *Pre-miR27a*- Polymorphism- Thai breast cancer

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Introduction

Breast cancer is the most common cancer of women worldwide. In Thailand, the tumor in 40.3% of breast cancer patients spread to lymph node and 23.4% of cases have distant metastasis (Hospital based cancer registry, Medical record and database cancer unit, Medical Digital Division, National cancer Institute, Thailand, 2020). The etiology of breast cancer derived from environmental factors and genetic susceptibility. Researches on candidate genes have been investigated in order to understand breast cancer pathogenesis, with an emerging interest in epigenetics and gene regulation.

MicroRNAs (MiRNAs) are one of the most important mechanism of gene regulation. MiRNAs are non-coding single stranded RNA of 22 nucleotides that regulate gene expression by blocking translation of mRNA. MiRNAs play an important role in many molecular pathway and biological process including cell proliferation, cell cycle control, apoptosis, cell differentiation and metabolism (Jansson and Lund, 2012). A single miRNA can target hundreds of mRNA and about 50% of *miRNA* genes are located in cancer related chromosomal region. MiRNA may participate in carcinogenesis, progression and prognosis of human cancer. *MiRNA* polymorphism has been reported to affect miRNA processing and miRNA-mRNA interaction. Aberrant expression of *miRNA* may affect the etiology, diagnosis and prognosis of cancer (Ma et al., 2013). Single nucleotide polymorphism (SNPs) in gene coding for pre-miRNA can be used as biomarkers for breast cancer susceptibility and prognosis (Wang et al., 2013).

There is evidence that miR27a plays an important role in carcinogenesis, cell proliferation, apoptosis, invasion, migration and angiogenesis. Moreover, miR27a has clinical significance in drug sensitivity, treatment and prognosis of cancer (Li et al., 2019). Recently, many studies have identified an important role of *pre-miR27a* (rs895819) A>G polymorphism in several types of cancer. In Thailand, most of the breast cancer patients are diagnosed in late stage and the disease progression into the stage of poor prognosis. Therefore, the purpose of this research is to investigate the association of *pre-miR27a* (rs895819) A>G and breast cancer susceptibility, prognostic factors

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and survival.

Materials and Methods

Specimen

The retrospective blood samples comprised of 143 breast cancer patients and 100 normal individuals were collected from National Cancer Institute, Thailand. All cases had Thai ethnicity with age ranged from 26 - 77 years (Mean ± SD) (51.43 ± 10.56). Clinicopathological data of breast cancer cases included histological grading, tumor size, tumor staging, lymph node involvement, distant metastasis. Immunohistochemistry result of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and triple negative status of ER-negative, PR-negative and HER2-negative were collected from pathology laboratory of National Cancer Institute of Thailand. The study was approved by the Ethics committee of the National Cancer Institute and inform consent was obtained from all the participants (Code number 031_2020RB_OUT67).

DNA extraction.

Genomic DNA in 100 healthy controls and 143 breast cancer patients was isolated from EDTA blood using High Pure PCR Template Preparation kit (Roche Molecular Diagnostics, Mannheim, Germany). The DNA purity and concentration was determined by using spectrophotometer measurement of absorbance at 260 and 280 nm. The concentration of DNA was 50 ng in total volume PCR reaction of 25 µL. The purity of DNA calculated by absorbance of 260/280 ratio is 1.8-2.0

Genotyping of pre-miR27a (rs895819) using PCR-RFLP

Genotyping of *pre-miR27a* (rs895819) was investigated in genomic DNA using polymerase chain restriction length polymorphism (PCR-RFLP) as described previously (Sun et al., 2010). The 182-bp DNA fragment containing the polymorphic site was amplified using 5′-GAA CTT AGC CAC TGT GAA CAC CAC TTG G-3′, and 5′-TTG CTT CCT GTC ACA AAT CAC ATT G-3′ as forward and reverse primer, respectively. The PCR cycling conditions were the initial denaturation of 95°C for 5 min followed by 35 cycles of 95°C for 35 sec, annealing at 60°C for 30 sec, and 72°C for 30 sec, with a final elongation at 72°C for 6 min. The PCR products were identified on electrophoresis on 2% agarose gel. The PCR product sizes, after restriction digested with *DraII* (New England Bio-Labs, USA) at 37°C overnight, were assessed on electrophoresis on 2.5% agarose gel and stained with NEOgreen (GELLGENTEK Co., Ltd. Korea). The restriction fragments included a single 182 bp fragment for the GG genotype which is homozygous polymorphism, two fragments of 155 and 27 bp for the AA genotype which is wild type; and three fragments of 182, 155 and 27 bp for the AG genotype which is heterozygous polymorphism.

The quality control of genotyping was done by DNA sequencing in 10% of cases and the result of sequencing is the same as the result of PCR-RFLP.

Statistical analysis

The statistics were based on two tailed probabilities and a p-value of <0.05 was considered statistically significant. The differences of age between 143 breast cancer patients and 100 control subjects were assessed by Student's T-test. The allele frequencies of *pre-miR27a* (rs895819) genotypes were calculated from Hardy–Weinberg equilibrium. The association between *pre-miR27a* (rs895819) genotypes and risk of breast cancer susceptibility was assessed by Pearson Chi-square and odds ratios (OR) with 95% confidence intervals (CI). Carriers of the genotype AA comprised of the reference group. The association between *pre-miR27a* (rs895819) genotype frequencies and clinicopathological data in breast cancer cases was evaluated by Pearson Chi-square test. The association between *pre-miR27a* (rs895819) genotype frequencies and overall survival was investigated by Kaplan–Meir method and log rank test. The Cox regression method was utilized to assess the prognostic effect for *pre-miR27a* (rs895819) A>G on breast cancer patient survival. The P <0.05 was considered a significant association.

Results

Pre-miR27a (rs895819) genotypes were analyzed by restriction digestion with *DraII* enzymes in 143 breast cancer cases and 100 normal controls. The age range of all cases were from 26 to 77 years age (Median age, 52 years). There was no statistically significant difference with mean age between breast cancer patients (51.43+4.56) and normal control. (51.39+4.55), (P = 0.975).

Table 1. Distribution of *pre-miR27a* Genotypes in 143 Breast Cancer Cases and 100 Controls

Genotype	Case N=143	Control N=100	OR (95%CI)	P-value
pre-miR27a rs895819				
AA	33	21	1 (Reference)	
AG	75	69	0.69, (0.37-1.31)	0.257
GG	35	10	2.23 (0.091-5.44)	0.078
AG+GG	110	79	0.89, (0.48-1.64)	0.701
Allele frequency				
A allele	0.49	0.55		
G allele	0.51	0.45		

Significance P-value < 0.05; OR, odds ratio; CI, Confidence interval

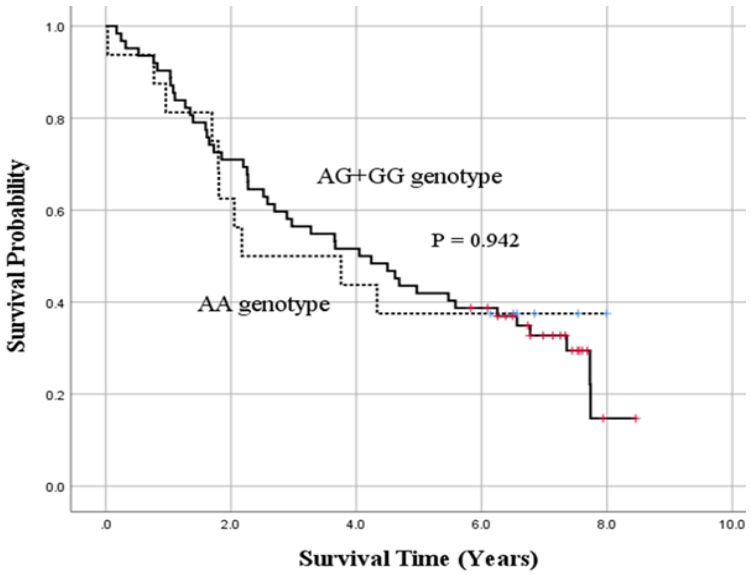

Figure 1. The Survival Curves were Analyzed by Kaplan–Meier Method and log Rank Test was Used to Compared the Survival Time between AG+GG Genotype Compared with AA Genotype. (P=0.942)

Table 2. Association of *pre-miR27a* Genotype and Clinicopathologic Data of Breast Cancer Patients

Clinicopathological data	Number	Genotypes		OR (95% CI)	P-value
		AA	AG or GG		
Age (Year)					
≤ 50	62	15	47	1.117	0.782
>50	81	18	63		
Tumor size (cm)					
≤ 3	96	21	75	0.687	0.387
>3	38	11	27		
Histologic grade					
I+II	53	20	33	3.196	0.006*
III	69	11	58		
Tumor stage					
I, II	69	15	54	0.940	0.886
III, IV	57	13	44		
Lymph node					
Positive	73	16	57	1.160	0.723
Negative	57	14	43		
Estrogen receptor					
Positive	75	14	61	0.510	0.098
Negative	58	18	40		
Progesterone receptor					
Positive	87	15	72	0.355	0.011*
Negative	46	17	29		
HER2					
Positive	97	25	72	1.438	0.448
Negative	36	7	29		
Triple negative					
Yes	78	24	54	2.611	0.031*
No	55	8	47		
Distant metastasis					
No	97	20	77	0.623	0.419
Yes	17	5	12		

Significant P-value < 0.05; OR, Odds Ratio; CI, Confidence Interval; Triple negative is ER negative, PR negative and HER negative

Association of pre-miR27a (rs895819) genotypes in breast cancer cases and controls

The distribution of *pre-miR27a* (rs895819) genotypes in 143 breast cancer cases and 100 controls was shown in Table 1. There was no association between *pre-miR27a* (rs895819) AG genotype (OR = 0.69, p-value = 0.257), GG genotype (OR = 2.23, p-value = 0.078), and AG+GG genotype (OR = 0.89, p-value = 0.701) genotype with breast cancer susceptibility (Table 1). The allelic frequency for *pre-miR27a* (rs895819) genotypes for both study populations were calculated.

Association of pre-miR27a (rs895819) genotypes and clinicopathological data

The association of *pre-miR27a* (rs895819) genotypes and clinicopathological data of 143 cases of breast cancer cases was assessed by Pearson Chi-square. It was found that miR27a (rs895819) variant genotype (AG and GG genotype) was significantly associated with poorly differentiated breast cancer (Grade III), (P = 0.006). Moreover, *miR27a* (rs895819) polymorphism (AG and GG genotype) was statistically associated with PR positive (P = 0.011) and triple negative breast cancer cases (P = 0.031) (Table 2).

Survival of breast cancer patients

Overall survival analysis was determined by Kaplan-Meir survival curve and Log rank test which was used to compare the survival time between *pre-miR27a* (rs895819) variant genotype (AG and GG genotype) and AA genotype of breast cancer patients.

It was found that there was no statistically significant difference of overall survival time in *pre-miR27a* (rs895819) variant genotype (AG and GG genotype) and AA genotype of breast cancer patients, (P= 0.942) as shown in Figure 1. Furthermore, the multivariate Cox regression method of prognostic marker for survival of breast cancer patients revealed that *pre-miR27a* (rs895819) A>G genotype was not an independent prognostic factor

Table 3. Multivariate COX Regression Methods of Prognostic Marker for Survival of Breast Cancer Patients

Clinical variables	HR	OR	P-value
Age; >50 vs ≤50	0.845	0.40-1.78	0.658
Tumor size (cm); >3 vs ≤ 3	0.467	0.07-2.98	0.421
Histologic grade; III vs I+II	1.457	0.68-3.12	0.331
Tumor stage; III vs I,II	4.251	1.74-10.36	0.001*
Lymph node invasion; negative vs positive	1.943	0.73-5.18	0.184
Triple negative tumor; ER/PR/HER2 negative vs ER/PR/HER2 positive	1.901	0.68-5.34	0.223
pre-miR27a rs895819; AG+GG vs AA	1.209	0.46-3.15	0.698

*Significant p-value < 0.05; HR, Hazard ratio; OR, Odds Ratio; CI, Confidence Interval

that affect the survival of breast cancer.

However, multivariate Cox regression analysis revealed that Tumor Stage III was prognostic biomarker that affect breast cancer survival when compare with stage I and II, (P =0.001) (Table 3).

Discussion

MicroRNAs are small non-coding RNA, which play an important role in controlling cancer development by regulating target genes at post transcriptional level. (Bartel, 2004). MicroRNA27 family composes of miR27a and miR27b. MicroRNA27a play an important role in tumor development by controlling genes involved in cell proliferation, apoptosis, differentiation, cell cycle regulation and chemotherapeutic resistance. MiR27a has been first found in breast cancer as an oncogenic function because high miR27a expression increased the percentage of cells in G2/M stage. (Mertens et al., 2007). MiR27a has rendered not only oncogenic function in breast cancer, lung cancer, hepatocellular carcinoma, prostate cancer, but also acts as tumor suppressor gene in gastric cancer, bladder cancer and esophageal squamous cell carcinoma (Li et al., 2019). Moreover, miR27a regulates the tumor immune response and epithelial mesenchymal transition (Zhang et al., 2019).

The presence of SNPs in the *miR* gene may involve in its expression process in cancers. There are two sites of polymorphism at *pre-miR27a* region of *miR27a* gene including rs895819 and rs11671784 located at positions 40 and 36 related to the first nucleotide (Gong et al., 2015). The *pre-miR27a* (rs895819) A>G polymorphism has been first identified. This mutant genotype increased the production of mature miR27a (Li et al., 2019). It was reported that *pre-miR27a* (rs895819) polymorphism is significantly associated with increased risk of lung cancer susceptibility but not in gastric cancer and esophageal cancer (Chen et al., 2017, Shankaran et al., 2020). The inconsistent results on rs895819 polymorphism in colorectal cancer have been reported (Chen et al., 2017, Zhang et al., 2020, Shankaran et al., 2020). The present case-control study found that *pre-miR27a* (rs895819) AG and GG genotype was not associated with breast cancer susceptibility. (P = 0.701). This present finding concurred with the study of Liu et al., 2021, who investigated the meta-analysis on the association of *pre-miR27a* (rs895819) polymorphism and breast cancer susceptibility. The results showed that no significant association between *pre-miR27a* (rs895819) polymorphism and breast cancer susceptibility in Asian or Chinese subpopulations. Nevertheless, they found that among Caucasians, the wild-type AA genotype at rs895819 may confer increased susceptibility to breast cancer, while the G-allele and AG genotype may be the protective factors. A study by Zhang et al. demonstrated that the G-allele exhibited decreased in risk of breast cancer in the younger Chinese women (Zhang et al. 2013).

Inconsistent results on the possible relationship between *pre-miR27a* (rs895819) polymorphism and clinicopathological parameters have been reported in Chinese population. One report showed that no significant association was observed between *pre-miR27a* (rs895819) polymorphism and clinicopathological parameters, including pathologic grade, TNM stage, estrogen and progesterone receptors, HER-2 status (Qi et al, 2015). In contrast with findings of another study done by Zhang et al. found significant association between rs895819 polymorphism and histological grade and estrogen status in older age group with moderately associated with progesterone status (Zhang et al. 2013). In this study, we found that *pre-miR27a* (rs895819)A>G is statistically significant associated with poorly differentiation (P =0.006), progesterone receptor (P= 0.011) and triple negative cases (P = 0.031) (Table2). Consistent with the prognostic indicator for breast cancer observed by Zhang et al. (Zhang et al. 2013), our result also indicated that rs895819 polymorphisms was not associated with overall survival of breast cancer cases. As positive for triple hormone receptor negative status and low grade tumor were both associated with rs895819 polymorphisms, therefore, *pre-miR27a* rs895819 A>G may be served as a candidate biomarker of poor prognosis for breast cancer in Thai population.

Author Contribution Statement

All authors contributed equally in this study.

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the supervision.

Ethical Declaration

The ethical issue was approved by ethical committee of National Cancer Institute (Code number 031_2020RB_OUT67).

Conflict of Interest

There is no conflict of interest.

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