RESEARCH ARTICLE

Role of *Estrogen Receptor Alpha* rs3798577 Polymorphism in Breast Carcinoma Risk Determination

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Abstract

BACKGROUND: Interaction between estrogen and estrogen receptor (ER) takes part in the regulation and differentiation of breast tumorigenesis. Some *ERα* polymorphisms, including *ERα* rs3798577, are reported to be associated with the risk and aggressiveness of breast carcinoma since the site was reported to be targeted by microRNA, which can further modulate the *ERα* expression. Hence, this study was conducted to disclose the possible role of *ERα* SNP rs3798577 on breast carcinoma patients.

METHODS: Samples were taken from the post-mastectomy breast carcinoma tissues of female patients and screened based on the completeness of medical and histopathological records. DNA isolation was proceeded using real time-polymerase chain reaction (RT-PCR) then analyzed for high resolution melting (HRM). The nucleotide base sequence was then analyzed based on rs3798577 *ERα* polymorphism. ER immunohistochemistry test was carried out and counted quantitatively based on the staining intensity and the percentage of the stained cells.

RESULTS: Out of 65 samples, there were 33 samples as wild type and 32 samples as variant type. Most variant and wild type had >80% *ERα* percentage. Most variant type had middle *ERα* intensity, while wild type had strong *ERα* intensity. Higher percentage of variant type (52.2%) was found with weak *ERα* histoscore, meanwhile higher percentage of wild type (52.4%) was found with strong *ERα* histoscore, but not significant (p=0.725).

CONCLUSION: *ERα* rs3798577 variant type had a lower *ERα* intensity and weaker *ERα* histoscore compared to the wild type, suggesting that *ERα* rs3798577 polymorphism might play a role in breast carcinoma risk determination.

KEYWORDS: breast cancer, *ERα*, rs3798577, polymorphism, immunoexpression

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Introduction

Breast carcinoma remains as the most prevalent malignancy in women worldwide, in line with its morbidity and mortality rate.(1,2) Incidence of breast carcinoma has increased each year and becomes the leading cause of cancer deaths in more than 100 countries.(3) The incidence and mortality varied among countries.(4) Frequent incidence was found on women in the South East Asia region.(5) In 2013, the

number of breast carcinoma in Indonesia was 98,692 cases and the total number in Sumatera Barat was 2,285 cases.(6) When early-stage breast carcinoma is diagnosed, the 5-year survival rate might increase up to 99%. Furthermore, tumor-free condition is mostly experienced by the majority of breast carcinoma survivors for the rest of their lives.(7)

Interaction between estrogen and estrogen receptor (ER) results in cell growth stimulation in several different tissues, including in breast epithelial tissue.(8,9) ER takes a part in the regulation and differentiation of normal breast epithelial cells as well as in breast tumorigenesis. Estrogen also plays a role in the genetic instability, through the DNA damage due to free radicals and mutation.(10)

In humans, ER alpha (*ERα*)/estrogen receptor 1 (*ESR1*), is located at chromosome 6, locus 6q25.(11) *ERα* is a target of immunohistochemical regular examination (12), which is correlated with better prognosis and the likelihood of response against the anti-estrogen therapy.(13) Some of the main strategies, including estrogen deprivation and the utilization of selective estrogen degradator, show effectiveness in managing the disease either in the advanced or the early stage. However, the clinical resistance related to the disease development remains a significant therapy challenge.(14)

Breast cancer therapy is categorized based on the subtype, determined by expression of ER, progesterone receptor (PR) and human epidermal growth factor receptor (HER)-2. Triple negative of ER/PR/HER-2 demonstrated aggressive and bad prognosis due to unresponsiveness to HER-2 and hormonal targeted therapies. In Indonesian triple negative cases, subjects were mostly having large tumor size, lymphatic gland metastasis, high pathological level and relatively young ages.(15) However, genetic variation exists in triple negative cases that is proven by its wide intrinsic molecular variation and varied survival rate reported.(16)

Several *ERα* polymorphisms are known to be associated with the risk and aggressiveness of breast carcinoma.(17,18) *ERα* genetic variance plays an important role in the transcription and expression of protein.(19) At the moment, more than 50% of genetic tendencies are the single nucleotide polymorphisms (SNP) and their roles remain unexplainable.(20) One of the *ERα* SNP, reference sequence (rs)3798577, was reported to be associated with the increase of breast carcinoma risk.(21) *ERα* SNP rs3798577 was reported in the population of Asia.(22) Although the SNP rs3798577 position is not within the functional domain of *ERα*, it was reported to bind with the microRNA-targeted site, which appeared to take a part in modulating the *ERα* expression.(23) To our knowledge, the study of *ERα* SNP rs3798577 on the cancer patients of Indonesian, especially in Sumatera Barat, has not been conducted yet. Therefore, current study was conducted to disclose the possible role of *ERα* SNP rs3798577 on breast carcinoma patients in Sumatera Barat.

Methods

Sample Retrieval and Selection

Frozen and paraffin embedded post-mastectomy breast carcinoma tissues of female patients operated in Djamil, Ropanasuri Hospital or Yarsi Ibnusina Hospital in 2015-2017 were retrieved and screened based on the completeness of medical and histopathological records. This protocol was approved by the Research Ethics Committee, Faculty of Medicine Universitas Andalas (No. 100/KEP/FK/2020).

DNA Isolation, High Resolution Melting (HRM) Analysis and Sequence

DNA isolation and purification of frozen post-mastectomy breast carcinoma tissues was performed at the Biomedical Laboratory, Faculty of Medicine, Universitas Andalas, using GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA). The isolated DNA was proceeded using real time-polymerase chain reaction (RT-PCR) with the previously reported forward primer for *ERα*: 5'-CCTGAACTTGCAGTAAGGTCA-3' and reverse primer: 5'- CCACCCTGAGCAAGTCT-3' (21), then analyzed for HRM using SensiFAST HRM Kit (Meridian Bioscience, Cincinnati, OH, USA) at Genomic and Molecular Breeding Laboratory, Faculty of Agricultural Technology, Universitas Andalas. After HRM analysis, several clusters were selected for sequencing examination at the 1st BASE Laboratory in Seri Kembangan, Selangor, Malaysia. The nucleotide base sequence was analyzed based on rs3798577 with BioEdit (BioEdit, Manchester, UK) and ChromasPro softwares (Technelysium Pty Ltd, South Brisbane, Australia).

ERα Immunohistochemistry and Analysis

The immunohistochemistry was carried out at Laboratory of Anatomic Pathology, Faculty of Medicine, Universitas Padjadjaran/Hasan Sadikin Central General Hospital, Bandung. Briefly, tissue paraffin blocks were 4-µm sliced, deparaffinized, antigen-retrieved, washed with phosphate buffered saline (PBS), blocked with 3% hydrogen peroxide and 2% bovine serum albumin. Then Estrogen Receptor

(EP1) Rabbit Monoclonal Primary Antibody (Cell Marque, Rocklin, CA, USA) was applied on the tissue section. After that, Starr Trek Universal Horseradish Peroxidase (HRP) Detection System (Biocare Medical, Pacheco, CA, USA) was used, resulting in a brown reaction product. Finally, the section was counterstained with hematoxylin and mounted. For analytical purposes, in each immunohistochemically stained tissue section, the fields were documented and quantitatively assessed by two observers according to the staining intensity and the stained cells percentage. The intensity was scored as 0 (negative), 1 (weak), 2 (intermediate), or 3 (strong), while the percentage of the stained cell was scored as 0 (negative), 1 (<20%), 2 (20%-50%), 3 (>50%-80%), 4 (>80%). In addition, histoscore was also performed, the immunohistochemical results were categorized into weak expression (histoscore: 0-5) and strong expression (histoscore: 6-12).(24)

Results

Seventy-five samples were collected, but 10 of them could not be amplified. From RT-PCR results, the remaining 65 samples were amplified as shown in the amplification graph with a cycle threshold value of 20-27 (Figure 1). These 65 samples were originated mostly from the subjects aged >50 years old (67.7%). Further analyses with high resolution melting (HRM) and sequencing were performed, and 2 large clusters were discovered. One of the clusters was *ERα* wild type, no alteration of the base and the other one was *ERα* variant type. Based on NC_000006.12 database, there was only 1 point of T>C substitution, located at 152,099,995. There were 33 samples (50.8%) as wild type and 32 samples (49.2%) as variant type. From the electropherogram, the wild type was homozygote and the variant type was heterozygote (Figure 2).

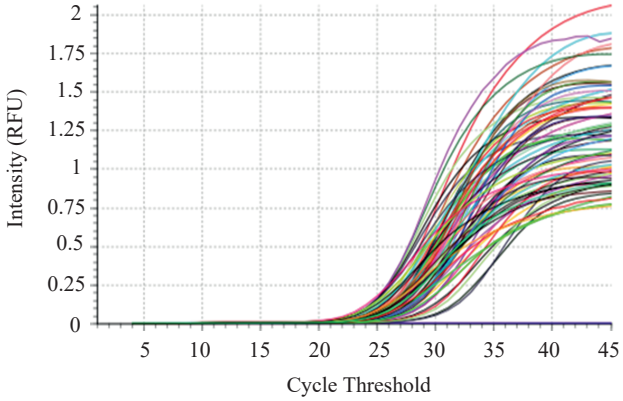

Figure 1. Amplification graph of RT-PCR for *ERα* with a cycle threshold value of 20-27.

ERα$^+$ cells were clearly expressed as brown stained cells (Figure 3). Distribution of *ERα* immunohistochemical expression and genotype could be observed in Table 1. Most variant as well as wild type had >80% *ERα* percentage. In terms of *ERα* intensity, mostly variant type had middle *ERα* intensity, while wild type had strong *ERα* intensity. Higher percentage of variant type (52.2%) was found with weak *ERα* histoscore, meanwhile higher percentage of wild type (52.4%) was found with strong *ERα* histoscore (Table 2), but not significant (Chi-square test, p=0.725)

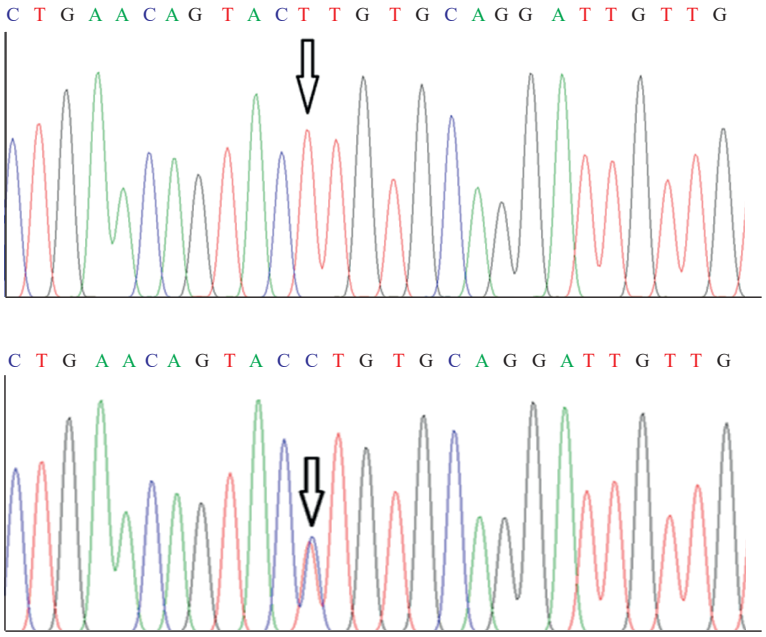

Figure 2. Electropherogram of the 2 clusters of *ERα*. A: wild type with homozygote genotype; B: variant type with heterozygote genotype.

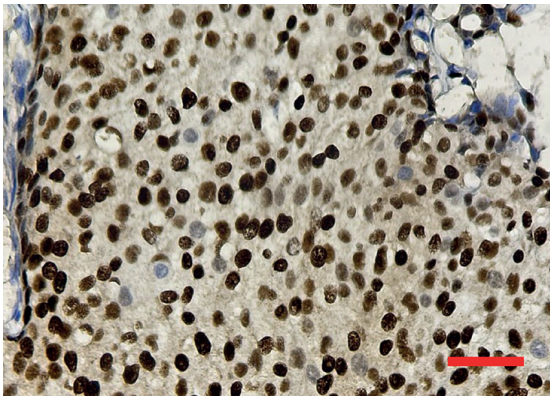

Figure 3. Immunohistochemical expression of *ERα*. Red bar: 100 µm.

Discussion

ERα rs3798577 SNP has been reported to play important roles in post-transcription regulation, nucleocytoplasmic mRNA transport, translation efficiency, sub-cellular localization (25) and stability (26). Current results showed that *ERα* rs3798577 variant type had a lower *ERα* intensity as well as weaker *ERα* histoscore, than the wild, although not significant. Based on an *ERα* RT-PCR and *ERα* immunohistochemistry study on breast carcinoma patients, the mRNA expression level had shown high conformance with the *ERα* histoscore.(27) Therefore, current results suggested that the *ERα* rs3798577 with T>C substitution, located at 152,099,995, was more correlated in reduction of the mRNA expression level and the ER histoscore.

Quantitative histochemical measurement with histoscore, especially the score of ER/PR/HER-2, has been recommended as a more precise predictor for the mortality risk of breast carcinoma.(28) A study of histological assessment and genotyping in the Tunisian population demonstrated that *ERα* rs3798577 and *ESR2* rs1256049 were related to the ER^+ breast carcinoma cases.(29) However, ER^+ immunohistochemical staining does not always show an active ER.(30) *ERα* mutations also do not appreciably diminish ER^+ staining by immunohistochemistry.(31) A study in Jordanian-Arab population population shows that *ERα* rs3798577 polymorphism was significantly associated with high risk and history of breast carcinoma.(32) In the American population, all assessed *ERα* variants, including rs3798577 are associated with the risk of breast carcinoma. (33) However, a study based on histopathological and genotyping analyses in the Korean population, rs3798577 decreased the risk in overall patients. For the premenopausal patients <35 years, *ERα* rs3798577 polymorphisms lowered the risk of breast carcinoma. rs3798577 decreased the risk in luminal B and triple-negative subtypes.(18) Therefore, the relationship between *ERα* rs3798577 polymorphism and breast carcinoma is still controversial.

Not only related to *ERα* merely, *ERα* rs3798577 as well as rs2234693 and rs3020314 were associated with breast carcinoma in HER-2-negative cases.(29) Additionally, *ERα* rs3798577 is also correlated with PR expression but the difference in survival could be related to the association between this polymorphism with PR status.(21)

There are several variants other than rs3798577 that have been reported to be associated with breast carcinoma. A three stage genome-wide association study reported that SNP rs2046210, which is located at 6q25.1 and upstream of *ERα*, showed consistent and strong association with breast cancer risk.(34) In a recent study, three variants of *ERα*, *i.e.*, rs9397437, rs3757322 and rs2747652 were associated with the risk of all breast cancer subtypes, namely triple-negative, HER-2-positive/non-luminal, luminal A-like, luminal B-like/HER-2-negative, and luminal B-like/ HER-2-positive. The strongest associations were observed

Table 1. Distribution of samples based on *ERα* immunohistochemical expression and *ERα* genotype.

Parameter	Genotype		n
	Variant	Wild	
***ERα* Percentage**			
<20%	1	1	2
20-50%	9	9	18
>50-80%	9	4	13
>80%	13	19	32
***ERα* Intensity**			
Weak	6	3	9
Middle	16	14	30
Strong	10	16	26
Histoscore			
Weak	12	11	23
Strong	20	22	42

Table 2. Correlation of *ERα* genotype with *ERα* histoscore.

Genotype	Histoscore		*p*- value
	Weak n (%)	Strong n (%)	
Variant	12 (52.2%)	20 (47.6%)	0.725
Wild	11 (47.8%)	22 (52.4%)	

between both rs9397437 and rs3757322 and the risk of triple-negative subtypes, as well as between rs2747652 and the risk of HER-2$^+$ subtypes.(35)

Study reporting the gene polymorphism of *ERα* rs3798577 has not been conducted in Indonesia previously. In the current study, although not significant, reduction of *ERα* intensity and low ER histoscore were found in *ERα* rs3798577. Therefore, further study should be conducted with a greater number of subjects. More investigations are also needed to identify the association between other *ERα* variants and ER$^+$ breast cancer cases. The development of combination therapies targeting canonical pathways that interchange with ER should be conducted to obtain better clinical responses and survival, such as cell-cycle and Phosphoinositide 3-kinases (PI3K) pathway inhibitors to improve clinical outcomes in the combination with anti-estrogen therapy.(36) It has been suggested that combination of a Bcl2 inhibitor, an anti-estrogen (tamoxifen/AI/fulvestrant) and an MDM2 inhibitor might be potential for treating ER$^+$ breast carcinoma.(37)

Conclusion

Results of the current study shows that *ERα* rs3798577 variant type had a lower *ERα* intensity as well as weaker *ERα* histoscore compared to the wild type. This suggests that *ERα* rs3798577 polymorphism plays a role in breast carcinoma risk determination.

Authors Contribution

PK was involved in the conceptualization and design of the study, data collection, and manuscript drafting. PK and FS interpreted the data and edited the manuscript. WAH, BN, IW, RK, BSH, and FS contributed to giving ideas and critical revision for the manuscript. All authors discussed the results and commented on the manuscript.

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