RESEARCH ARTICLE

E-cadherin and Vimentin as Predictors of Resistance to Preoperative Systemic Therapy in Patients with Advanced Breast Cancer

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Abstract

Loss of E-cadherin and increased vimentin expression are associated with epithelial-mesenchymal transition and cancer stemness which are responsible for treatment resistance. The study aims to evaluate the role of E-cadherin and vimentin as predictors of resistance to preoperative systemic therapy in patients with advanced breast cancer. This was a cross-sectional analytical study in patients with stage III-IV breast cancer in Dharmais Cancer Hospital and dr. Cipto Mangunkusumo National Hospital from July 2015 to April 2016. Patients had biopsy specimens embedded in paraffin blocks. Expressions of E-cadherin and vimentin proteins were done immunohistochemically. Treatment response was evaluated histopathologically using Miller-Payne criteria on mastectomy specimens. A total of 65 patients were enrolled. Five patients with invasive lobular carcinoma were excluded. Thirty one had chemotherapy and 29 had hormonal therapy. After treatment, 46 patients were eligible for mastectomy. E-cadherin and vimentin were positive in 28 (60.9%) and 11 (20.3%) of specimens. Twenty-three (50%) patients showed no response. Treatment resistance were associated with type of therapy (OR=4.4; 95% CI=1.27-15.41; p=0.017) and vimentin expression (OR=6.75; 95% CI=1.27-30.02; p=0.016). Hormonal therapy (OR_{adj}=6.26; 95%CI=1.59-24.6; p=0.009) and positive vimentin (OR_{adj}=8.75; 95%CI=1.43-57.4; p=0.019) were independent predictors of treatment resistance. In conclusion, E-cadherin and vimentin can be used as predictors of resistance to preoperative systemic therapy in patients with advanced breast cancer.
Keywords: *breast cancer, cancer stemness, E-cadherin, preoperative therapy, vimentin.*

Peran E-cadherin dan Vimentin sebagai Prediktor Resistensi Terapi Sistemik Preoperatif pada Pasien Kanker Payudara Stadium Lanjut

Abstrak

Hilangnya ekspresi E-cadherin dan meningkatnya ekspresi vimentin dihubungkan dengan epithelial-mesenchymal transition dan cancer stemness yang bertanggungjawab terhadap resistensi terapi sistemik preoperatif. Tujuan studi ini untuk mengevaluasi peran E-cadherin dan vimentin sebagai prediktor resistensi terapi sistemik preoperatif pada kanker payudara stadium lanjut. Studi analitik dengan desain cross sectional ini dilakukan di RS Kanker Dharmais dan RSUPN dr. Cipto Mangunkusumo sejak Juli 2015 sampai April 2016. Pasien kanker payudara stadium III-IV dibiopsi dan sampel dibuat blok parafin. Ekspresi E-cadherin dan vimentin dianalisis secara imunohistokimia. Respons terapi dievaluasi secara histopatologi dengan kriteria Miller-Payne pada pasien yang dilakukan mastektomi. Terdapat 65 pasien namun 5 pasien karsinoma lobuler dieksklusi. Sebanyak 31 pasien diberikan kemoterapi dan 29 pasien diterapi hormonal; setelah terapi, 46 pasien layak mastektomi. E-cadherin dan vimentin positif pada 28 (60,9%) dan 11(20,3%) spesimen. Dua puluh tiga (50%) pasien tidak menunjukkan respons terhadap terapi. Resistensi terapi dihubungkan dengan tipe terapi (OR=4,4; 95% CI=1,27-15,41; p=0,017) dan ekspresi vimentin (OR=6,75; 95% CI=1,27-30,02; p=0,016). Terapi hormonal (OR_{adj}=6,26; 95%CI=1,59-24.6; p=0,009) dan ekspresi vimentin (OR_{adj}=8,75; 95%CI=1,43-57,4; p=0,019) adalah prediktor independen resistensi terapi. Disimpulkan E-cadherin dan vimentin dapat berperan sebagai prediktor resistensi terapi sistemik preoperatif pada pasien kanker payudara stadium lanjut.
Kata kunci: *kanker payudara, cancer stemness, E-cadherin, terapi preoperatif, vimentin*

Introduction

Breast cancer is the most common cancer among women worldwide, including Indonesia, and accounted for 25% of all new cancers diagnosed in 2012.[1]Advanced stage breast cancer consists of locally advanced breast cancer (LABC) or stage III and metastatic breast cancer (MBC) or stage IV.[2,3] These groups of patients are still commonly found in Indonesia; 41% and 22% of new cases in Dharmais Cancer Hospital were stage III and IV disease, respectively.[4]

Treatment optionsfor advanced stage breast cancer are limited and resistance frequently occurs.Standard treatment for inoperable, non-inflammatory LABC is pre-operative chemotherapy with anthracylin-based regimen with or without taxane.[5] Pre-operative systemic treatment in inoperable LABC allows breast conserving surgery in some patients,[6] or alternatively, modified radical mastectomy.[7] Systemic treatment for MBC could prolong survival and improve the patient's quality of life, but not curative. The benefit of mastectomy in MBC patients is still controversial. Meta-analysis from 10 retrospective studies showed better 3-year survival rate (30%) compared to systemic treatment alone (22%).[8]In addition, primary tumor resection for MBC patients may give palliative benefits such as control of bleeding, ulceration or infection.[9]

Despite aggressive therapy, some patients showed treatment resistance and disease progression.[10]Resistance to treatment might be caused by the epithelial-to-mesenchymal transition (EMT) process.[11,12]EMT is a mechanism by which a solid tumor acquires metastatic capability. Normal breast epithelium expressed epithelial cadherin (E-cadherin) that contributes to intercellular junction. In advanced stage, epithelial-mesenchymal plasticity is increased and cancer cellsmay convert from epithelial to mesenchymal phenotype expressing vimentin.[13,14]The EMT process is reversible because the mesenchymal cellscan undergo dedifferentiation back into epithelial phenotypeand re-expressed E-cadherin.[15]Cancer cells with mesenchymal phenotype acquire stem cell characteristics and called cancer stem cell (CSC).[16] These cells are resistant to treatment and may cause disease progression.[17,18]Loss of cell-adhesion proteins (such as integrin and E-cadherin) and the emergence of vimentin expression are hallmarks of EMT process. The final result is increased cell survival and resistance to therapy.[19,20]This study aims to evaluate the association of E-cadherin or vimentin expression and preoperative systemic treatment response.

Method

Study Design and Subjects

This was an analytical cross-sectional study on advanced stage breast cancer patients in Dharmais Cancer Hospital and Dr. Cipto Mangunkusumo National Hospital from July 2015 to April 2016. Patients were included if they had pathologically confirmed breast cancer with clinical stage III-IV according to TNM system. All patients had biopsy specimens before preoperative systemic therapy. Patients underwent systemic treatment either with chemotherapy or hormonal therapy. Chemotherapy regimens consisted of 5-fluorouracil, adriamycin, and cyclophosphamide (FAC) given for 6 cycles; hormonal therapy were given surgically (bilateral salpingo-oophorectomy) or medically using *selective estrogen receptor modulators* (SERMs) or *aromatase inhibitors* (AIs) in hormonal positive receptor patients.

E-cadherin and Vimentin Expressions

Expressions of E-cadherin and vimentin proteins were assessed using immuno-histochemistry staining on paraffin sections of biopsy specimens. A 4µm thick section was cut and mounted on anobject glass. Antigen retrieval and staining was performed with an autostainer (Ventana BenchMark GX, Roche). E-cadherin staining was done using monoclonal mouse anti-E-cadherin/CDH1 antibody (clone 4A2C7, TermoFisher Scientific, USA) whereas monoclonal rabbit anti-vimentin antibody (RMAB 019 clone SP20, DiagnosticBiosystem, USA) was used to stain vimentin protein. Slides were left on the tray for 30 minutes for antibody incubation. Afterwards, slides were washed under running water for 5 minutes and were dehydrated using ethanol series in an increasing concentration, i.e. 70%; 96% and absolute ethanol for 5 minutes each. A threshold value of 5% was used to define positive expression of E-cadherin and vimentin. Slides without specific primary antibodies served negative controls.

Evaluation of Treatment Response

Evaluation of treatment response was done histopathologically on mastectomy specimens using Miller-Payne criteria as follows:[21]

- Grade 1: no change or some alteration to individual malignant cells but no reduction in overall cellularity.
- Grade 2: A minor loss of tumor cells but overall cellularity still high; up to 30% loss.
- Grade 3: Between an estimated 30% and 90% reduction in tumor cells.

- Grade 4: A marked disappearance of tumor cells such that only small clusters or widely dispersed individual cells remain; more than 90% loss of tumor cells.
- Grade 5: No malignant cells identifiable in sections from the site of the tumor; only vascular fibroelastotic stroma remains often containing macrophages. Ductal carcinoma in situ may be present.

For statistical analysis, treatment response were grouped as no response (Miller-Payne grade 1) and complete or partial response (Miller-Payne grade 2-5).

Statistical Analysis

Clinical characteristics of the study subjects were presented descriptively as frequency and percentage. The association between preoperative systemic treatment and clinicopathological variables was tested using chi-square of Fisher's exact test. A p value of less than 0.05 was considered significant. Logistic regression analyses were undertaken to identify the best combination of risk factors for treatment resistance. The adjusted odds ratio (OR_{adj}) and 95% confidence interval (CI) were estimated using the logistic regression coefficient. Analyses were performed using statistical software (IBV SPSS version 21, SPSS Inc., USA).

Results

A total of 65 patients were enrolled during the study period with a mean age of 47.9±10.25 years. There were 5 cases with invasive lobular carcinoma which were excluded from the analysis. After treatment, 46 patients were eligible for modified radical mastectomy, which included 29/32 (90.6%) stage IIIB and 17/28 (60.7%)stage IV patients (Figure 1). All specimens were evaluated for histopathological response; however, only mastectomy specimens were included for statistical analysis Table 1).

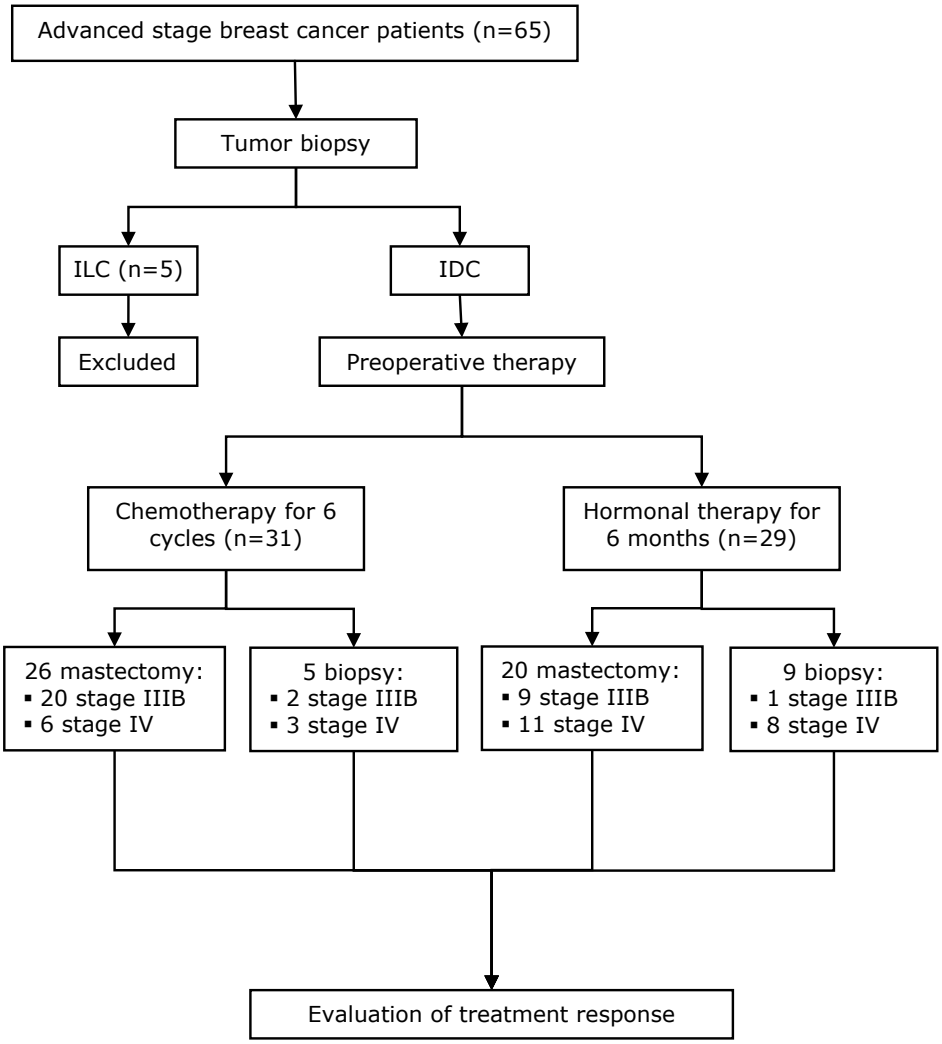

Figure 1. Study Flow Chart

Table 1. Characteristics of the Study Subjects (n=65)

Variables	n	%
Age group		
≤35 years	8	12.3
>35 years	57	87.7
Stage		
Stage IIIB	34	52.3
Stage IV	31	47.7
Histopathological type		
Invasive ductal carcinoma	60	92.3
Invasive lobular carcinoma	5	7.7
Histopathological grade		
Grade 1	11	16.9
Grade 2	29	44.6
Grade 3	25	38.5
Molecular subtypes		
Luminal A	41	63.1
Luminal B	11	16.9
HER2-positif	6	9.2
Triple negative	7	10.8
E-cadherin expression		
Positive	40	61.5
Negative	25	38.5
Vimentin expression		
Positive	18	27.7
Negative	47	72.3

Expression of E-cadherin and Vimentin

Among 46 patients eligible for statistical analysis, positive E-cadherin was found in 28 (60.9%) cases, while positive vimentin expression was observed in 11 (23.9%) cases. There was neither association between E-cadherin expression and clinicopathological factors nor between vimentin expression and clinicopathological factors (Table 2).

Table 2. Association between E-cadherin or Vimentin Expressions and Clinicopathological Variables (n=46)

Variables	E-cadherin			Vimentin		
	Positive	Negative	p	Positive	Negative	p
Stage						
Stage IIIB	18 (62.1%)	11 (37.9%)	0.828*	8 (27.6%)	21 (72.4%)	0.501#
Stage IV	10 (58.5%)	7 (41.2%)		3 (17.6%)	14 (82.4%)	
Grade						
Grade 1	6 (85.7%)	1 (14.3%)	0.220#	3 (42.9%)	4 (57.1%)	0.333#
Grade 2-3	22 (56.4%)	17 (43.6%)		8 (20.5%)	31 (79.5%)	
Subtype						
Luminal	20 (55.6%)	16 (44.4%)	0.274#	7 (19.4%)	29 (80.6%)	0.220#
Non-luminal	8 (80.0%)	2 (20.0%)		4 (40.0%)	6 (60.0%)	

*Chi-square test; #Fisher's exact test

Evaluation of Treatment Response

Mastectomy specimens were available from 46 cases; 23 (50%) among them had no response to preoperative therapy. Hormonal therapy (OR 4.4) and vimentin expression (OR 6.75) were significantly associated to more failure of preoperative treatment (Table 3). Expression of E-cadherin tended to be a protective factor towards non-responsive treatment. On multivariate analysis (Table 4), hormonal therapy and positive vimentin expression were identified as independent predictors for non-responsive treatment.

Table 3. Factors Associated with Response to Preoperative Therapy (n=46)

Variables	NR	CR/PR	p	OR	95% CI
Stage					
Stage IIIB	15 (51.7%)	14 (48.3%)	0.760*	1.205	0.363-3.998
Stage IV	8 (47.1%)	9 (52.9%)			
Grade					
Grade 1	6 (85.7%)	1 (14.3%)	0.096#	7.765	0.852-70.752
Grade 2-3	17 (43.6%)	22 (56.4%)			
Subtype					
Luminal	19 (52.8%)	17 (47.2%)	0.475*	1.676	0.403-6.966
Non-luminal	4 (40.0%)	6 (60.0%)			
Preoperative therapy					
Hormonal	14 (70.0%)	6 (30.0%)	0.017*	4.407	1.260-15.414
Chemotherapy	9 (34.6%)	17 (65.4%)			
E-cadherin					
Positive	12 (42.9%)	16 (57.1%)	0.227	0.477	0.143-1.597
Negative	11 (61.1%)	7 (38.9%)			
Vimentin					
Positive	9 (81.8%)	2 (18.2%)	0.016	6.750	1.265-30.029
Negative	14 (40.0%)	21 (60.0%)			

*Chi-square test; #Fisher's exact test; NR: no response; CR: complete response; PR: partial response

Table 4. Multivariate Analysis to Predict Treatment Resistance

Variables	β	SE	p	OR_{adj}	95% CI
Hormonal therapy	1.73	0.69	0.009	6.26	1.59 – 24.6
Positive vimentin	2.26	0.92	0.019	8.75	1.43 – 57.4
Constant	-3.07				

SE= standard error; OR_{adj}=adjusted OR; CI: confidence interval

Discussion

This study was the first comprehensive study in Indonesia on advanced stage breast cancer patients assessing the expressions of E-cadherin and vimentin proteins and their predictive role on preoperative treatment response. Despite the advanced nature of the disease, less than 40% of biopsy specimens in the current study showed negative E-cadherin expression, including three cases of ILC type. In further analysis, ILC was excluded because loss of E-cadherin protein in this histopathology type is mostly due to mutation of E-cadherin gene promoter, CDH1.[22,23]However, among the rest IDC type, positive E-cadherin cells are still high, suggesting intact intracellular adhesions and retaining epithelial phenotype of the cells.On the other hand, vimentin expression was observed in less than 30% patients of all IDC type patients. However, positive vimentin expression was not corresponded to negative E-cadherin.

Systemic therapy is the mainstay of treatment in advanced breast cancer, which includes chemotherapy, hormonal (endocrine therapy), and targeted therapy.Benefit of neo-adjuvant chemotherapy is not limited to down-stage the tumor only but also to select next treatment based on pathological treatment response and to choose the next treatment.[24]In addition, neo-adjuvant endocrine

treatment is a logical option for postmenopausal patients with endocrine response (strongly positive hormone receptor, low proliferation).[25]

In this study, patients were given preoperative systemic treatment followed by a mastectomy whenever clinically possible. More than 90% of stage IIIB and 60% of stage IV patients underwent mastectomy, giving an overall rate of 76.7% for successful down-staging. However, only 50% of them showed some degree of histopathological response (Miller-Payne grade 2 to 5). Evaluation of residual tumor using Miller-Payne criteria was based on reduced tumor cellularity between biopsy at diagnosis and mastectomy specimens after preoperative systemic therapy.[21]Previous study in Pathology Department, dr. Cipto Mangunkusumo Hospital, found that 35.7% of 42 LABC patients were not responsive to neo-adjuvant chemotherapy.[26]The rate of pathological complete response (pCR) varies according to the molecular subtype of breast cancer.[27,28] Pathological CR is related to better survival after neo-adjuvant chemotherapy and surgery.[29]

Bivariate analysis showed that treatment response was significantly associated with type of treatment and vimentin expression. Both variables consistently showed significant association in multivariate analysis. Treatment resistance could be predicted by hormonal therapy and positive vimentin.

Resistance to hormonal therapy can occurs in all stages, but the most difficult cases are in the recurrence setting. Although resistance may exist in about half patients before treatment, it alsomay occur during treatment.[30]There are several mechanisms involved in hormonal therapy resistance, such as mutation of ER gene (ESR1), epigenetic aberration, and signaling crosstalk.[31]ESR1mutation, especially at the ligand-binding domain(LBD) site, seems to be the major mechanism of resistance related to AI therapy in metastatic breast cancer.[32,33]The resistance may not be identified at the time of diagnosis, but it emerges due to selective pressure of multiple endocrine therapy. Additionally, genetic instability occurring at an advanced stage could also contribute to mutation rate, for example due to defect of DNA repair mechanisms which remain due to the selective pressure.[34]

Conclusion

A considerably high number of patients with advanced stage breast cancer showed positive E-cadherin but low vimentin expressions (27.7%) suggesting an early process of epithelial-mesenchymal transition. E-cadherin and vimentin can be used as predictors of resistance to preoperative systemic therapy in patients with advanced breast cancer.

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