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Association of Dual Specific Phosphatase 4 (DUSP4) Expression and Anthracycline-Based Neoadjuvant Chemotherapy Response in Breast Cancer

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Abstract

Background: Chemotherapy is important component in the management of breast cancer. To achieved better chemotherapy response, a predictive marker is needed. Various studies shows that DUSP4 expression correlates with chemotherapy response. Objectives: Aims of this study is to know association between DUSP4 expression with anthracycline-based neoadjuvant chemotherapy in locally advanced breast cancer. Methods: This is an observational study with longitudinal study method, DUSP4 expression is confirmed with Immunohistochemistry, chemotherapy response is calculated using RECIST criteria. Results: Total sample of this study were 63 breast cancer patients. This study shows that there is a tendency for better chemotherapy response in DUSP4 expression, although it is not statistically significant (p=0.073). Stratification of DUSP4 expression analysis based on intrinsic subtype, DUSP4 expression correlated with luminal B subtype (p=0.024), OR : 2.33 (1.106 - 4.492).

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DUSP4 expression on other subtypes were no significantly correlated, luminal A (*p=0.245*) and Her2 (*p*=0.612). Conclusion: Overall DUSP4 expression is less associated with chemotherapy response. DUSP4 expression on subtype Luminal B has a significant association with chemotherapy response.

Keywords: Breast Cancer; Chemotherapy; Clinical Response; DUSP4; Intrinsic subtype; Anthracycline.

1. Introduction

Breast cancer is the most frequent cancer in women in the world, incidence approximately 1,380,000 and mortality 458,400 cases every year [1]. Incidence in Indonesia estimated 48,998 breast cancer every year, most of them present in advanced stage [2, 3].

Neoadjuvant chemotherapy has become a standard in advanced stage breast cancer management [4]. Neoadjuvant chemotherapy offers many advantages [5]. Anthracycline-based chemotherapy is most often used in breast cancer chemotherapy [6].

Anthracycline has long term accumulated toxicity: cardiac dysfunction, myelodysplasia and leukemia [6, 7]. Anthracycline also has limitation in breast cancer related to drug resistancy [8, 9].

Breast cancer patient with the same staging dan given with the same chemotherapy regiment doesn't always has the same outcome. To achieved better clinical response, it is needs a predictive marker on breast cancer [10]. An ideal biomarker should differ various response to certain chemotherapy agent, before a chemotherapy procedure has done [5, 11].

Study on residual breast cancer sample patient after neoadjuvant chemotherapy, found polymorphisms of DUSP4 on residual tumor [12]. DUSP4 is an enzyme which inactivates kinase target through dephosphorylase phosphoserine residual and phosphotyrosine [13-15]. DUSP4 acts as ERK phosphatase. Inhibition of ERK phosphatase reduces tumor sensitivity to chemotherapy.

DUSP4 correlated negatively with MEK-ERK pathway. DUSP4 expression can predict MEK-ERK activity. Chemotherapy will be more effective if DUSP4 is expressed [12, 16, 17].

Because of the importance of predictive marker in management of breast cancer, we are interested to study the DUSP4 expression in breast cancer with chemotherapy response. The objective of this study is to know the association between DUSP4 expression with Anthracycline-based chemotherapy response in locally advanced breast cancer patients.

2. Materials and Method

2.1. Collection of Samples

This is an observational study with longitudinal study model. Study was conducted within a population of breast cancer patients who had been diagnosed through clinical and histopathology examination, which entered

the Wahidin Sudiro Husodo Hospital in Makassar, South Sulawesi, Indonesia, starting from October 2014 to September 2015.

Inclusion criteria are locally advanced breast cancer female patient, invasive ductal breast cancer, receiving neoadjuvant chemotherapy with CAF (Cyclophophamide, Adriamycine, 5-FU) regiment. Exclusion criteria were inadequate tissue samples for immunohistochemistry and bilateral breast cancer.

All samples who fulfilled inclusion and exclusion criteria and willing to participate in the study and signing informed consent recruited as study samples. Total samples of this study were 63 breast cancer patients.

2.2. Expression DUSP4 by Immunohistochemistry

DUSP4 expression is measured with immunohistochemistry as described by Hyunsung Kim and his colleagues 2015. DUSP4 expression is evaluated from its coloring intensity and size.

Coloring intensity is scored : negative (0), weak (18), medium (2) and strong (3), and coloring size is scored: 0% (0), 1-25% (18), 26-50% (2), 51-75% (3) and 76-100% (4). Final score was results of Intensity times the coloring score. DUSP4 expression is classified as negative (score 0-3) and positive (score 4-12). [19, 20]

2.3. Classification of clinical response to chemotherapy

Chemotherapy response is classified as nonresponsive, if tumor size is reduced ≤ 30%, no change or increased in tumor size, or if found a new tumor; while responsive, if tumor is disappear, or there is a reduction >30% and no new tumor found.

2.4. Data Analysis

Data analysis using the SPSS (Statistical Package for Social Science) version 22. Analysis of patient's characteristics and clinical response using chi square.

2.5. Ethical Clearence

Ethical approval for this study was obtained from Research Ethics Committee, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. Number; 581/H4.8.4.5.31/PP36-KOMETIK/2015.

3. Results

In this study we have 63 breast cancer samples, the youngest was 29 years old and the oldest one was 75 years old, the most age in population was the fourth decades 22/63 (35%). Histopathologic we got *Low Grade* 9 cases (14.3%), *Moderate Grade* 33 cases (52.4%) dan *High Grade* 21 cases (33.3%).

Immunohistochemistry panel we got Luminal A 14 (22.2%), Luminal B 26(41.3%), Her2 18 (28.6%) dan Triple Negative 5 (7.9%). DUSP4 was expressed in 21/63 (33.3%). Responsive to neoadjuvant chemotherapy CAF

regiment was 35/63 (55.6%). Characteristic of samples can be see in the following table 1.

Table 1: Clinicopathological characteristics

Characteristic	Number (Percentage %)
AGE	
< 50	43 (68.3%)
≥ 50	20 (31.7%)
GRADE	
Low Grade	9 (14.3%)
Moderate Grade	33 (52.4%)
High Grade	21 (33.3%)
SUBTYPE	
Luminal A	14 (22.2%)
Luminal B	26 (41.3%)
HER2	18 (28.6%)
Triple Negative	5 (7.9%)
DUSP4	
Positive	21 (33.3%)
Negative	42 (61.7%)
CLINICAL RESPONSE	
Responsive	35 (55.6%)
Non-responsive	28 (44.4%)

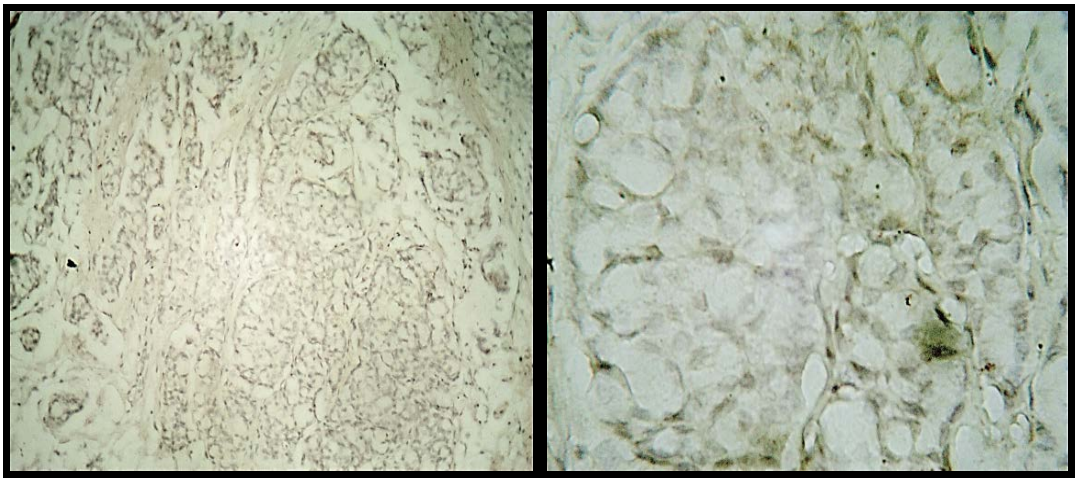

Figure 1: Microphotographs of Dual Specific Protein Phosphatase 4 (DUSP4) immunostaining in invasive ductal carcinoma. Negative Results in 40 x and 400 x

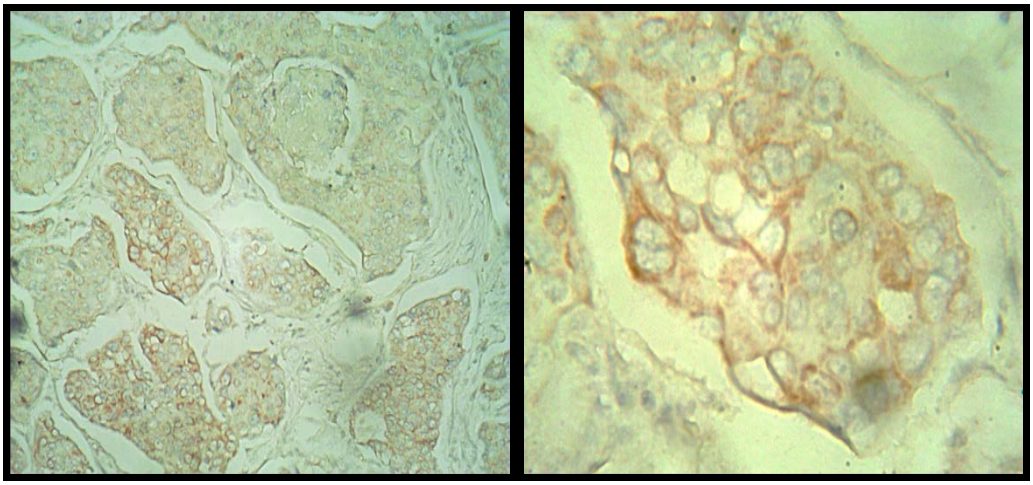

Figure 2: Microphotographs of dual-specificity protein phosphatase 4 (DUSP4) immunostaining in invasive ductal carcinoma. Positive results in 40 x and 400 x. The tumor cells showed cytoplasmic DUSP4 staining.

Table 2: Association between DUSP4 expression with clinical response to neoadjuvant Chemotherapy

DUSP4	Clinical Response		Total	*p*	OR (95%CI)
	Responsive	Nonresponsive			
Positive	15 (71.4%)	6 (28.6%)	21(33.3%)	0,073	1.5 (0.98 –2.27)
Negative	20 (47.6%)	22 (52.4%)	42(66.7%)		

DUSP4 expression has a tendency to affect chemotherapy response in breast cancer, it can be seen that positive DUSP4 tends to be responsive to neoadjuvant chemotherapy 15/21 (71.4%), and negative DUSP4 tends to be non-responsive to neoadjuvant chemotherapy 22/42 (52.4%).

However, there is no significant difference statistically with *p value* = 0.073 ($p>0.05$. It means that DUSP4 expression is less associated with Anthracycline-based neoadjuvant chemotherapy

Stratisfication of breast cancer based on intrinsic subtype, we got DUSP4 expression in Luminal A *p* value = 0.24 (> 0.05) and in Her2 *p value* = 0.61 (> 0.05). It means that DUSP4 expression in subtype Lumina A and Her2 are not associated with chemotherapy response in breast cancer.

In subtype Luminal B, there are difference in clinical response between DUSP4 positive expression 10 (38.5 %) dan DUSP4 negative expression 5 (19.2%), with *p value* = 0.021 ($p<0.05$).

Value *crude odds ratio* (21) = 2.3 with *confidence interval* (95% CI) = (1.16 – 4.92). It means that DUSP4 expression in subtype Luminal B is associated with Anthracycline-based neoadjuvant chemotherapy.

Table 3: Correlation Between Subtype, DUSP4 with Chemotherapy Response

SUBTYPE	DUSP4	CHEMOTHERAPY RESPONSE		*p*	*OR (95%CI)*
		Responsive	Nonresponsive		
		n (%)	n (%)		
LUMINAL A	DUSP4 Positive	3 (75)	1 (25)	0.245	2.5 (0.83 – 7.52)
	DUSP4 Negative	3 (30)	7 (70)		
LUMINAL B	DUSP4 Positive	10 (83.3)	2 (16.7)	0.021	2.3 (1,16 – 4,92)
	DUSP4 Negative	5 (35.7)	9 (64.3)		
HER2	DUSP4 Positive	2 (40.0)	3 (60.0)	0.608	1.5 (0.57 – 4.20)
	DUSP4 Negative	8 (61.5)	5 (38.5)		
TRIPLE NEGATIVE	DUSP4 Positive	0 (0)	0 (0)		
	DUSP4 Negative	4 (80)	1 (20)		

4. Discussion

Result of this study revealed that positive DUSP4 expression shows tendency for a better chemotherapy response compared to negative DUSP4 expression, but it is not statistically significant. DUSP4 expression on Luminal B subtype is associated with chemotherapy response.

Study on DUSP4 expression is still rare. Study on breast cancer profile shows expression of DUSP4 on 39/115 (34%) sample, from DUSP4 which is expressed on protein level, 29 samples with gen amplification [22]. Other studies found that low DUSP4 expression is correlated to basal-like breast cancer, high tumor proliferation after chemotherapy, lack of response to chemotherapy and shortening of disease free survival. In contrast, over-expression of DUSP4 is correlated to increasing apoptosis induced by chemotherapy [12, 16]. Other study found that low DUSP4 expression correlated to increasing recurency rate and mortality in triple negative breast cancer patients [23]. Study found that high DUSP4 expression needs a higher doxorubicin dosage, while cells with low DUSP4 expression need a lower doxorubicin dosage [8]. Study with other agent shows that DUSP4 also acts in regulating breast cancer cell sensitivity for other chemotherapy agents [8].

Study mentions that DUSP4 expression was significantly correlated with a larger tumor size (>2 cm, p=0.015). There was no significant correlation between overall survival or disease-free survival and DUSP4 expression in all 266 patients [19].

The limitations of this study were; Neoadjuvant chemotherapy is only done three cycles, which aims to downsizing until the tumor becomes resectable, so that in this study no cases were found to achieve pCR (pathological complete response). Measurement of dependent variable were using the manual method by using a caliper, ideally using a measuring instrument such as CT SCAN / MRI.

5. Conclusion

DUSP4 expression shows tendency of a better chemotherapy response, but it is not statistically significant. DUSP4 expression on Luminal B subtype has a significant correlation with chemotherapy response. Examination of DUSP4 Expression with immunohistochemically methods should be considered in patients with Breast Cancer mainly in Luminal B Subtypes undergoing chemotherapy, to obtain better treatment outcomes. Based on this study, it can be suggested for further study of DUSP4 expression in breast cancer.

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6. Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare

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