CELL SURFACE HER2/neu ONCOPROTEIN EXPRESSION IN BREAST DUCTAL CARCINOMA

Imam Susilo
Department of Anatomic Pathology
Airlangga University School of Medicine
Dr Soetomo Teaching Hospital, Surabaya

ABSTRACT

Breast cancer is a malignant tumor mostly disclosed in women. It has heterogeneous biological behavior - so that the knowledge of tumor markers is very important to determine its prognosis and therapy. Up to now, the determination of prognosis and treatment of choice is still based on clinical and morphologic finding although recent studies pointed out that there was tight relationship between carcinoma growth and molecular abnormalities including normal cell gene consisting of proto-oncogene, tumor suppressor gene, programmed cell death and DNA repair gene. Therefore, the description of molecular changes is required - in determining the prognosis and therapy of breast cancer. Molecular pathologic approach may offer a prospective promise even though the genetic mechanism of molecular carcinogenesis of breast cancer is still unclear. In this study, immunopathologic investigation was carried out by using immunohistochemical method, with antibody monoclonal against protein HER2/neu. Based on multivariate test of Wilks' Lambda method, protein expression of HER2/neu was concomitantly different in various tumor diameters of breast cancer ($p = 0.000 < \alpha = 0.05$). With method of Wilks' Lambda method, protein expression HER2/neu was simultaneously different in various carcinoma cell differentiation of breast cancer ($p = 0{,}000 < \alpha = 0.05$) and with Wilks' Lambda method, protein expression HER2/neu, was concomitantly different in various progressiveness of ductal carcinoma growth ($p = 0{,}000 < \alpha = 0.05$). Also with Wilks' Lambda method, protein expression HER2/neu was concomitantly different in various grade of ductal carcinoma ($p = 0.000 < \alpha = 0.05$). It showed that cancer of the breast occurred in genetic lesion.

Keywords: *Ductal carcinoma of the breast, prognosis, molecular pathological role*

Correspondence: Imam Susilo, Department of Anatomic Pathology, Airlangga University School of Medicine, Dr Soetomo Teaching Hospital, Surabaya

INTRODUCTION

Breast cancer is a malignant tumor commonly found in women. It has heterogeneous biological behavior, so that the determination of tumor marker becomes highly important to determine the prognosis and management of the disease (Indrawati et al. 2004). In addition to other factors, the prognosis of the disease is determined much by the progressiveness of the growth of tumor cell itself. The assessment of tumor progressiveness by observing cell proliferation activity to predict the prognosis of a neoplasmic disease is an important step in the effort to manage malignant disease, including breast ductal carcinoma. The observation can be directed to the morphology of cell and tissue, and also cell morphofunction that may indicate cancer biological activity.

Recently, breast ductal cancer can be morphologically differentiated into three groups, i.e., breast ductal carcinoma with low, moderate, and high histopathological grading (Kumar et al. 2005; Rosai 1996; Rosai 2004; Tavassoli & Devilee 2003). breast ductal carcinoma in low histopathological grading consists of relatively mature cancer cells that remains showing identifiable morphology as that of the original cells. High histopathological grading cancer consisted of immature cancer cells, which morphologically do not reflect specific shape and unidentifiable as normal breast ductal epithelial cells. Moderate histopathological grading cancer consisted of cancer cells that are in transition from low to high histopathological grading. Low histopathological grading breast ductal carcinoma has lower degree of malignancy compared to high histopathological grading. The progressiveness of tumor growth is indicated by the course of the disease from breast ductal carcinoma in situ, invasive, and metastatic. Metastatic breast ductal carcinoma is suggested to have higher cell proliferation activity as compared to those invasive or in situ (Tavassoli & Devilee 2003).

The morphological grouping of breast ductal carcinoma, however, remains having less significant clinical value, since such grouping has not been able to elaborate real grouping of breast ductal carcinoma based on the degree of cell proliferation activity (Kumar et al. 2005). It is

also unable to pathologically explain the molecular grade required to provide elaboration of the change of cancerf cell proliferation activity as the basis for determining the degree of malignancy, which is reflecting the biological activity condition of the cancer cells.

Every year, a hundred thousand novel patients are emerging, and in the United States thirty thousand women die from breast ductal cancer. It is the most common malignant disease in America and Europe (Rosai 1996, 2004). Up to now, high incidence and mortality rate due to breast cancer can still be found, either in the US or in Europe. It is the most common cancer found in women. In Indonesia, it occupies the second rank of malignancies in women after cervical cancer. Successfully disclosed cases are also increasing in number annually (National BRK 1995). The report of Directorate General of Medical Service, Indonesian Department of Health, along with Cancer Registration Board, Indonesian Association of Pathologist, and Indonesian Cancer Foundation in 1998, from 13 pathology centers in whole Indonesia, breast cancer holds the second rank (2598 cases) in Indonesia after cervical cancer (3682 cases), followed with tumors of lymph nodes, skin, and nasopharyngeal.

In Surabaya and its surrounding areas, from one year to another, breast cancer remains the second most common malignancy in women after cervical cancer. However, interesting findings can be found in several pathology centers, where breast cancer was found occupying the first rank in number, such as that in Medan (170 cases), Padang (120 cases), Palembang (113 cases), Yogyakarta (535 cases), and Makasar (147 cases). In Surabaya and its surrounding areas, it was reported that there were 400 to 750 breast cancer patients annually (Kusumowardojo et al. 2004). This cancer is commonly found in advanced stage with high degree of malignancy.

Data from the Department of Anatomic Pathology, Dr Soetomo Hospital, Airlangga University School of Medicine, showed that 43% of the patients are those with high malignancy and poorly differentiated cancer cells (Susraini 2001). Breast ductal carcinoma grouping to determine its malignancy and prognosis requires reliable scientific basis by disclosing the pathologic mechanism at molecular level pertaining to the biological activity of cell proliferation. Prognosis determination by grouping based on the morphology at cellular and tissue level, which is currently in use, apparently has not been able to explain the pathological mechanism of the biological activity of breast ductal carcinoma cell proliferation at molecular level. Such condition has lead to a notion that breast ductal cancer diagnosis and grouping still needs to be based on the pathological mechanism of cancer cell proliferation at molecular level, so that the abnormal control of proliferation and differentiation of breast ductal carcinoma cells can be disclosed. If such problem is left unnoticed, the result of diagnosis and grouping without knowing the molecular pathological mechanism of biological activity will be inapplicable, since it has less significant clinical value, particularly the prognostic determination and the choice of therapy, which may be disadvantageous for the patients.

Excessive proliferation of cancer cells, compared to dead cells, may raise growth fraction and enhance tumor growth, which is expected to grow more progressively. Until recently, high histopathological grading of breast ductal carcinoma is regarded as having worse course of disease, which is more progressive compared to that with low histopathological grading. Such condition occurs because high grade breast ductal carcinoma is assumed to have higher cell proliferation activity compared to those with lower histopathological grading (Kumar et al. 2005). Until recently, the identification of the activity of breast ductal carcinoma cells remains using molecular level pathological approach. Breast ductal carcinoma grouping with poor, moderate, or good differentiation have different cell proliferation activity. The mechanism of pathological changes at molecular level to disclose tumor progressiveness cannot be elaborated to determine the prognosis and management of the disease. Therefore, efforts to disclose the basic of activity mechanism of breast ductal carcinoma cell proliferation are needed to produce certain parameters of breast ductal carcinoma disease with expected clinical values through molecular pathology approach. The diagnosis determination of the parameter can be used as a basis for determining the management and evaluation, particularly to predict the disease's prognosis.

Various studies in the last decade indicate close correlation between growth progressiveness of a neoplasm and abnormality at molecular level. The existing of molecular abnormality or lesion cannot only be single, but also present as a highly complex genetical lesion accumulation. Basically, the genetical lesion accumulation can be grouped as tumor triggering gene (oncogene), gene suppressing tumor (GST) inactivation, and “Programmed Cell Death” (PCD) gene that leads to predominant oncogene. The predominant role and function of the oncogene may trigger excessive cancer cell proliferation and abnormal differentiation, since the activity of growth inhibition and programmed cell death is dysfunctioned (Yarnold 1996).

It can therefore be inferred that the assessment of the degree of tumor growth progressiveness based on histopathological grading and clinical stages still

requires basic elaboration at molecular level. If this effort is successful, it can be used to detect and evaluate abnormalities in controlling cell proliferation activity and to predict malignancy potential earlier, which has not been detected by morphological changes at cellular, tissue, or organ level.

In breast ductal carcinoma, the role of c-erb B2 coded by ErbB-2 oncogene is important (Yasasever et al. 2000). The role of c-erb B-2 protein expression may disturb the function of pRas and c-myc (proto-oncogene) as well as pRb (gene suppressor) in controlling cell proliferation and cycle (Ferdinal 2003). There is significant evidence that c-erbB-2 protein expression in breast ductal carcinoma cells is higher than that in benign lesion or breast ductal cells of healthy individuals (Strecfus et al. 2000). To molecular-pathologically disclose the change of cellular biological behavior change in the effort to predict the prognosis of breast ductal cancer, the expression of cell surface HER2/neu protein can be measured in various tumor diameter size, differentiation, growth progressiveness, and histopathological grading.

MATERIALS AND METHOD

This study was an observational analytical method using samples of tumor tissue from breast ductal carcinoma patients taken from the Department of Anatomic Pathology, Airlangga University School of Medicine, Dr Soetomo Teaching Hospital, Surabaya. In this study, we identified oncoprotein expression, reflecting biological behavior of breast ductal carcinoma, i.e., cell surface HER2/neu protein expression and tumor morphology regarding its diameter, differentiation (good, moderate, poor), growth progressiveness (in situ, invasive, and metastasis), and histopathological grading (low, moderate, high) of breast ductal carcinoma.

This study used cross-sectional design. The objects were cells and the tissue of breast ductal carcinoma tumor that had been operated at the Department of Surgery, Airlangga University School of Medicine, Dr Soetomo Teaching Hospital, Surabaya. To represent the population, sampling was performed using purpose sampling method. To obtain homogeneity, the samples were matched in age, sex, and homogeneity test.

The diagnosis of breast ductal carcinoma was evaluated routinely using histopathological swab examination with Hematoxylin-Eosin staining by examining cell morphology and structure, tissue histopathological profile, as well as the number of mitotic cells. These procedures were carried out by two senior pathologists, who had no information on the subjects' identity to establish the diagnosis of breast ductal carcinoma by determining the tumor diameter, differentiation, growth progressiveness, and histopathological grading, at the Department of Anatomic Pathology, Airlangga University School of Medicine, Dr Soetomo Teaching Hospital, Surabaya. The assessment of cell surface HER2/neu protein (oncogene receptor protein) expression was conducted at the Immunohistochemistry Division, Department of Anatomic Pathology, Airlangga University School of Medicine, Dr Soetomo Teaching Hospital, Surabaya, by a senior pathologist (consultant) who had competence in conducting such procedure. The evaluation cell surface HER2/neu gene oncoprotein expression was conducted using immunohistochemical method with monoclonal antibody. The subsequently emerging change of color marker label on the swab was evaluated semi-quantitatively.

Sample size was determined using Higgins formula (1985) as those in previous studies. Obtained sample size was 10. Since this study involved three groups, required samples consisted of 30 breast ductal carcinoma tissues.

RESULTS

Cell surface HER2/neu expression in various diameters of in situ breast ductal carcinoma (BDC)

Table 1. Cell surface HER2/neu expression in various diameters of in situ BDC

Tumor Diameter (cm)	HER2/neu Expression				Total (%)
	0	1	2	3	
≤ 2	2	-	-	2	4 (40 %)
$2 < x \leq 5$	2	-	-	-	2 (20 %)
> 5	2	-	-	2	4 (40 %)
Total	6	-	-	4	10 (100 %)

From 10 observed samples, it was found that the expression of cell surface HER2/neu protein of the tumor tissue cells in in situ BDC patients was varied. Positive expression was found in group with tumor size of less or equal to 2 cm (3+) and more than 5 cm (3+), each comprising 2 patients or 20%. BDC with expression of cell surface HER2/neu protein of 0 was found in all tumor diameter groups, each 2 patients or 20%.

Table 2. Protein expression of HER2-Neu Cell surface On Various Size Diameter KDPD Invasif

Tumor Diameter (cm)	HER2/neu expression				Total (%)
	0	1	2	3	
≤ 2	-	-	-	2	2 (20 %)
2 < x ≤ 5	4	-	-	2	6 (60 %)
> 5	-	-	1	1	2 (20 %)
Total	4	-	1	5	10 (100 %)

From 10 observed samples, it was found that cell surface HER2/neu protein expression in the tumor cell of invasive BDC patients were varied. Invasive BDC cases with positive cell surface HER2/neu protein were found in all tumor size groups, each comprising 2 patients or 20% in diameter group of ≤ 2 and 2 < x ≤ 5, with a score of 3+ and diameter group of > 5 cm comprised 2 patients or 20% with scores of 2+ and 3+. In this study, BDC with cell surface NER2/neu protein expression with score 0 was found in group with tumor size of 2 < x ≤ 5, comprising 4 patients or 40%.

Cell surface HER2/neu expression in various metastatic BDC diameters.

Table 3. Cell surface HER2/neu expression in various metastatic BDC diameters

Tumor diameter (cm)	HER2/neu expression				Total (%)
	0	1	2	3	
≤ 2	1	1	1	2	5 (50 %)
2 < x ≤ 5	1	-	1	1	3 (30 %)
> 5	1	-	-	1	2 (20 %)
Total	3	1	2	4	10 (100 %)

It was found that cell surface HER2/neu protein expression of the tumor tissue cell in BDC patients were metastatic. Metastatic BDC cases with positive cell surface HER2/neu protein expression were found in tumor diameter less or equal to 2 cm, comprising 3 cases (30%) in which 2 patients had score of 1+ and 2+ while 1 patient with score 3+, 2 < x ≤ 5 cm in 2 cases (20%), each 1 patients and more than 5 cm in 1 case or 10% with score 3+. BDC with cell surface HER2/neu protein expression with 0 score was found in all size groups, each 1 patient (10%). It was likely that excessive cell surface HER2/neu expression occurred in group with certain tumor diameter of < 2 cm.

Cell surface HER2/neu expression in various in situ BDC differentiations.

Table 4. Cell surface HER2/neu expression in various in situ BDC differentiations

Differentiation	HER2/neu Expression				Total (%)
	0	1	2	3	
Good	1	-	-	-	1 (10 %)
Moderate	-	-	-	-	-
Bad	5	-	-	4	9 (90 %)
Total	6	-	-	4	10 (100 %)

From the samples it was found that cell surface HER2/neu protein expression in tumor cell of BDC patients was varied. Cell surface HER2/neu protein expression in in situ BDC was found in well-differentiated group with score 0 in 1 patient or 10% of the cases. BDC group with poor differentiation was found in 5 cases with score of 0 (50%) and score 3+ in 4 cases (40%). In situ BDC cases with positive Cell surface HER2/neu protein expression was found in poorly differentiated tumor cases, comprising 4 patients (40%). BDC with negative cell surface HER2/neu protein expression was found in tumor group with good and poor differentiation, each comprising 1 and 5 patients or 10% and 50%. It was suggested that excessive Cell surface HER2/neu expression was found in certain tumor group, i.e., in that with poor differentiation.

Cell surface HER2/neu expression in various invasive BDC differentiations.

Table 5. Cell surface HER2/neu expression in various invasive BDC differentiations

Differentiation	HER2/neu Expression				Total (%)
	0	1	2	3	
Good	-	-	-	-	-
Moderate	1	-	1	4	6 (60 %)
Poor	3	-	1	-	4 (40 %)
Total	4	-	2	4	10 (100 %)

The samples showed that Cell surface HER2/neu protein expression in tumor cell of invasive BDC patients was varied. The cell surface HER2/neu protein expression in invasive BDC was found in moderate differentiation group with score 0, comprising 1 patient or 10% of the cases, score 2+ (1 patient or 10%) and score 3+ comprised 4 cases (40%). BDC groups with poor differentiation was found in 4 cases with score 0 (40%) and score 2+ in 1 case (10%). Invasive BDC cases with positive cell surface HER2/neu protein expression were found in tumor group with moderate and poor differentiation, each comprising 5 patients (50%) and 1 patient (10%), while BDC with negative cell surface HER2/neu protein expression was found in the same tumor group, comprising 1 and 3 patients or 10% and 30% of the cases. This indicated that excessive cell surface HER2/neu protein expression occurred in certain tumor group, than was in those with moderate differentiation.

Cell surface HER2/neu expression in various metastatic BDC differentiations.

Table 6. Cell surface HER2/neu expression in various metastatic BDC differentiations

Differentiation	HER2/neu expression				Total (%)
	0	1	2	3	
Good	-	-	-	-	-
Moderate	-	1	-	-	1 (10 %)
Poor	2	1	2	4	9 (90 %)
Total	3	2	1	4	10 (100 %)

From the samples, it was found that Cell surface HER2/neu protein expression in tumor cell of metastatic BDC patients was varied. Cell surface HER2/neu protein expression in metastatic BDC was found in group with moderate differentiation with a score of 1+ in 1 patient or 10% of the cases. BDC group with poor differentiation was found in 2 cases with score 0 (20%), score 1+ in 1 patient (10%) cases, score 2+ in 2 cases (20%), and score 3+ in 4 cases (40%). Metastatic BDC cases with positive cell surface HER2/neu protein expression was found in tumor group with poor differentiation in 6 patients (60%), BDC with negative Cell surface HER2/neu protein expression was found in 1 and 3 patients, or 10% and 30%. It was likely that excessive Cell surface HER2/neu expression occurred in tumor group with poorly differentiated cells.

Cell surface HER2/neu expression in various invasive BDC grading

Table 7. Cell surface HER2/neu expression in various invasive BDC grading

Histopathological grading	HER2/neu expression				Total (%)
	0	1	2	3	
Grade I	-	-	-	1	1 (10 %)
Grade II	1	-	-	3	4 (40 %)
Grade III	4	-	1	-	5 (50 %)
Total	5	-	1	4	10 (100 %)

From 10 observed samples, it was found that cell surface HER2/neu protein expression in tumor cell of invasive BDC patients was varied. Cell surface HER2/neu protein expression in invasive BDC was found in grade I with score 3+ in 1 case (10%), grade II with score 0 in 1 patient (10%) cases and score 3+ in 3 cases (30%). BDC group with grade III was found in 4 cases with score 0 (40%) and score 2+ in 1 case (10%). Invasive BDC cases with positive cell surface HER2/neu protein expression was found in tumor group with grades I, II, and III, each 1 (10%), 3 (30%), and 1 (10%) case. It was likely that excessive cell surface HER2/neu expression occurred in certain tumor group, i.e., the group with grade II.

Cell surface HER2/neu expression in various metastatic BDC grading

Table 8. Cell surface HER2/neu expression in various metastatic BDC histopathological grading

Histopathological grading	HER2/neu expression				Total (%)
	0	1	2	3	
Grade I	-	-	-	-	-
Grade II	-	1	-	-	1 (10 %)
Grade III	3	1	1	4	9 (90 %)
Total	3	2	1	4	10 (100 %)

From 10 observed samples, it was found that cell surface HER2/neu protein expression in tumor cell of invasive BDC patients was varied. Cell surface HER2/neu expression in metastatic BDC was found in grade II with score 1+ in 1 patient or 10% of the cases. BDC group with grade III was in 3 cases with score 0 (30%), with score 1+ in 1 patient (10%) of the cases, score 2+ was found in 1 cases (10%), and score 3+ was found in 4 cases (40%). Metastatic BDC cases with positive Cell surface HER2/neu protein expression was found in grade III tumor group, comprising 5 patients (50%). It was likely that excessive Cell surface HER2/neu expression was found in certain tumor group, the grade III BDC.

It was found that cell surface HER2/neu expression in various BDC diameter groups was significantly different. This was proved using Hotelling's Trace multivariate statistical test ($p = 0.000 < a = 0.05$). Figure 1 shows different mean of Cell surface HER2/neu protein expression in various BDC diameters, i.e., ≤ 2 cm (77.55), $> 2 - \leq 5$ cm (48.82), and > 5 cm (95.63) (Figure 1). It was also found that cell surface HER2/neu protein expression in various BDC tumor cell differentiations was significantly different, as proved from Hotelling's Trace statistical test with $p = 0.000 < \alpha = 0.05$. Figure 2 shows different mean of Cell surface HER2/neu protein expression in various in BDC tumor group in various differentiations, i.e, good (8.00), moderate (89.43), and poor (69.14) differentiations (Figure 2)

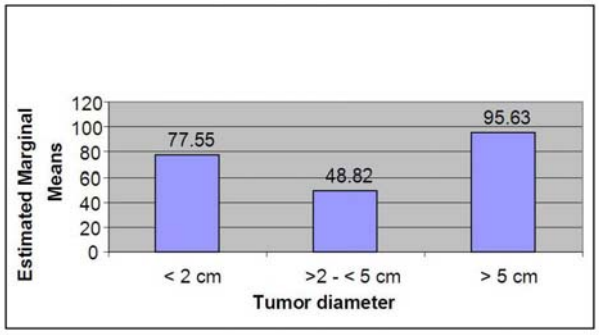

Figure 1. Cell surface HER2/neu protein expression in various BDC diameters

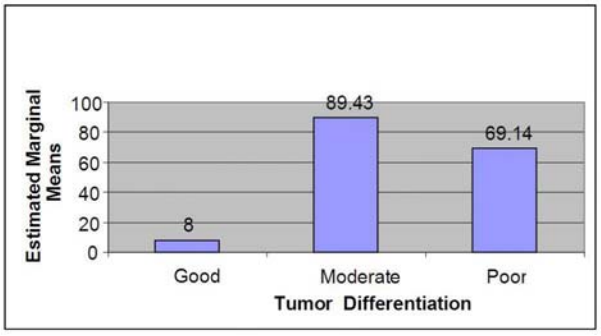

Figure 2. Cell surface HER2/neu protein expression in various BDC differentiations

This study revealed that cell surface HER2/neu protein expression in various BDC tumor growth progressiveness was significantly different. Using Hotelling's Trace multivariate test, it was found the value of $0.000 < \alpha = 0.05$. Figure 3 shows different mean of cell surface HER2/neu expression in various BDC tumor groups regarding tumor growth progressiveness, i.e., in situ (61.30), invasive (82.90), and metastatic (71.30) BDC (Figure 3). This study also revealed that cell surface HER2/neu protein expression in various BDC tumor histopathological grading groups was significantly different. The Hotelling's Trace revealed $p = 0.000 < \alpha = 0.05$. Figure 4 shows different mean of cell surface HER2/neu protein expression in various tumor histopathological grading, i.e, BDC grade I (116.00), KDPD grade II (70.40) and grade III (76.71) (Figure 4).

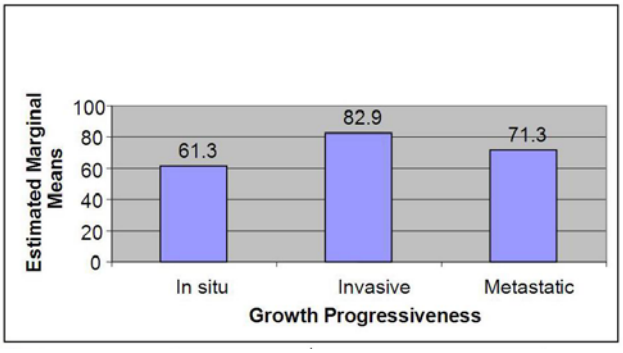

Figure 3. Cell surface HER2/neu protein expression in various BDC growth progressiveness

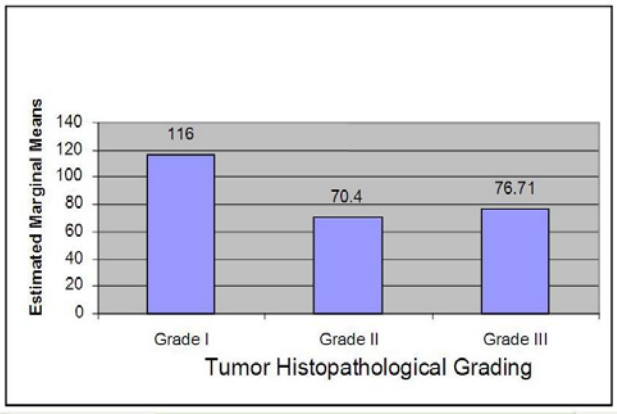

Figure 4. Cell surface HER2/neu protein expression in various BDC histopathological grading

DISCUSSION

The multivariate analysis of cell surface HER2/neu protein expression in various BDC tumor diameter indicates that cell surface HER2/neu protein expression was found in BDC of < 2 cm (mean 77.05), > 2 - < 5 cm (mean 48.82), and > 5 cm (mean 95.63). This indicates that the function of Cell surface HER2/neu receptor oncogene protein expression is important, since it is the first gate of extracellular stimulation pathway. The pathway has a role in controlling the growth of tumor cell altogether. In this study, statistical test revealed that cell surface HER2/neu protein expression in various BDC tumor diameters was different, as proved by multivariate statistical test, the Wilk's Lambda, with $p = 0.000 < \alpha = 0.05$, showing that all variables of cell surface HER2/neu protein expression were different in various BDC tumor diameter.

The absence of linear correlation between various oncoprotein expressions with BDC diameter had been addressed in previous report. A previous study has reported that age, tumor size, type of operation, chemoadjuvant therapy, adjuvant hormone therapy, and post-operative radiotherapy had insignificant p value in prognosis (Tsutsui et al. 2004). In selecting tumor marker, Cell surface HER2/neu receptor oncoprotein expression can be used as a biological parameter as it can be detected in BDC tumor with small diameter, and it can also be used as prognostic morphofunctional marker in small size tumor. Additionally, the cell surface receptor protein expression remains high in larger size BDC.

Multivariate analysis on cell surface HER2/neu protein expression in various BDC tumor differentiation revealed that cell surface HER2/neu protein expression in all tumor differentiation group relatively increased linearly, i.e., BDC with good (mean 8.00), moderate (mean 89.43), and poor (mean 69.14) differentiation. This shows that the function of Cell surface HER2/neu receptor oncogene protein expression is important in extracellular signal transmission that regulates cell cycle, whose pathway is vital in controlling cell growth. Cell activity, as reflected from tumor differentiation, particularly within the nucleus, indicates the presence of correlation with Cell surface HER2/neu protein expression.

Previous data showed that excessive expression of cell surface HER2/neu protein expression is related with histopathological grading and tumor cell differentiation. In this study, the excessive expression was 15 to 20% of primary breast cancer (Tsuda et al. 2005). This was because tumor differentiation reflects the activity of BDC tumor cell biological activity. Nevertheless, statistical test showed that cell surface HER2/neu protein expression in various BDC tumor differentiation altogether was different, as proved using Wilk's Lambda test of $p = 0.000 < \alpha = 0.05$. In this study, cell surface HER2/neu receptor oncoprotein expression can used as biological parameter since it can be detected in BDC with well-differentiated tumor, and it can also be used as tumor marker to determine the prognosis of BDC tumor. In addition to be detectable when the tumor remains well-differentiated, the cell surface protein receptor remains high in poorly-differentiated BDC tumor.

Multivariate analysis on cell surface HER2/neu protein expression in various BDC tumor growth progressiveness indicated that cell surface HER2/neu protein expression was high in almost all tumor growth progressiveness groups, i.e., in situ (mean 61.50), invasive (mean 82.90), and metastatic (mean 71.30) BDC. This demonstrates that the function of cell surface HER2/neu receptor oncogen protein determines the controlling process of tumor growth progressiveness. The protein is located in a pathway that has a role in controlling tumor cell growth. Cell activity, as reflected from the progressiveness of tumor growth, indicates positive correlation with cell surface HER2/neu protein expression.

Previous study also found that excessive expression from cell surface HER2/neu oncoprotein was found in 33% of in situ ductal cancer, and 60% of the incidence was detected in comedo-type of in situ ductal carcinoma. Similar finding was also found in this study, in which excessive expression of cell surface HER2/neu oncoprotein of in situ ductal carcinoma (33%) was higher than that in invasive ductal carcinoma (13%). However, this study was not performed in metastatic ductal carcinoma group (Tsuda et al. 2005). Such condition took place because growth progressiveness is reflecting the biological activity of BDC tumor cell. Therefore, it is plausible that the increase of extracellular ligand (GF family) stimulation and cell surface HER2/neu receptor oncoprotein expression may further enhance pRB phosphorilation process and MIB-1 protein expression. Nevertheless, statistical test revealed that cell surface HER2/neu protein expression in various BDC tumor growth progressiveness group altogether was different, as proved from Wilk's Lambda test with $p = 0.000 < \alpha = 0.05$. Cell surface HER2/neu protein was highly expressed in various growth progressiveness groups.

Multivariate analysis on the cell surface HER2/neu protein expression in various histopathological grading of BDC carcinoma revealed high expression in almost all tumor grade groups. Once again, it can be stated that

the function of cell surface HER2/neu receptor oncogene protein is important and responsible in cell cycle controlling pathway. Previous study also showed that excessive cell surface HER2/neu protein expression was found in in situ ductal carcinoma (33%), higher than that in invasive ductal carcinoma (13%). However, this study was not performed to metastatic ductal carcinoma group (Tsuda et al. 2005). Although statistical test showed that cell surface HER2/neu protein expression in various histopathological grading groups of BDC tumor altogether was different, as proved using multivariate statistical method of Wilk's Lambda with $p = 0.00 < \alpha = 0.05$. In this study, cell surface HER2/neu receptor oncoprotein expression can be used as biological parameter as it can be detected in low grade of malignancy of BDC, and can also be used as morphofunctional marker to determine the prognosis of tumor. Cell surface HER2/neu receptor oncoprotein expression can be detected in low grade tumor, and the cell surface receptor protein expression will remain high in tumor with moderate and high grade.

CONCLUSION

In conclusion, cell surface HER2/neu protein is expressed in significantly different ways in various tumor diameters (< 2 cm, > 2 cm to < 5 cm and > 5 cm), differentiation (good, moderate, and poor), growth progressiveness (in situ, invasive, and metastatic), and histopathological grading (low, moderate, and high) in breast ductal carcinoma (BDC), which present as its morphological and clinical profile. The growth process of BDC, as indicated by its diameter, differentiation, progressiveness, and histopathological grading, is the accumulated function of cell surface HER2/neu protein expression in tumor cells. Cell surface HER2/neu protein in breast ductal cancer, in addition to being excessively expressed, also undergoes structual and functional changes, which results in increasing cell biological activity. Cell surface HER2/neu protein expression can be used as morphofunctional, differential, as well as morphological marker in the attempt to determine appropriate prognosis and choice of therapy in breast ductal carcinoma (BDC).

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