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Relationship between Monocarboxylate Transporter-4 Expression and Breast Cancer Clinicopathology and Subtype in Sanglah General Hospital, Denpasar, Indonesia

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Abstract

BACKGROUND: Based on the global cancer observatory (GLOBOCAN) data, in 2018, there were 18.1 million new breast cancer cases. Breast cancer is the second-most common malignancy after lung cancer, which contributed to 11.6% of all new cancer cases in 2018. Breast cancer is the second-leading cause of death in women in developing countries. The activity of Warburg and reverse Warburg effects on breast cancer is reflected by the expression patterns of two molecules, namely caveolin-1 and monocarboxylate transporter-4 (MCT-4). MCT-4 is a transmembrane transport protein that functions in the transport of lactate from the cytoplasm to the intercellular fluid.

METHODS: This is a cross-sectional analytical study to determine the relationship between MCT-4 expression and breast cancer clinicopathology and subtypes. The study was conducted between April and May of 2020 with 62 breast cancer patients as samples in Sanglah General Hospital, Denpasar. Analysis was done with SPSS 25.

RESULTS: A logistic regression analysis was performed to analyze the relationship between the dependent variable (MCT-4) and covariates (stage, grade, and subtype). Of the three variables that were significantly associated with MCT-4 expression, only clinical stage and subtype (luminal and non-luminal) remained independently associated with MCT-4 expression. Analysis on the clinical stage and subtype variables showed an adjusted OR of 4.727 (p = 0.047; 95% CI: 1.109–21.922) and 17.850 (p = 0.009; 95% CI: 2.069–154.003), respectively. This suggests that MCT-4 has a significant association with subtype and clinical stage which increases the risk of progression of the cancer stage as well as the risk of developing a more malignant (non-luminal) subtype.

CONCLUSION: High MCT-4 expression was significantly associated with malignant subtypes, high histological-grade cancer, and an advanced breast cancer.

Introduction

Breast cancer is the second-most common malignancy in the women population and second only to lung cancer as the most common malignancy worldwide which contributes approximately 11.6% of all new cases in 2018. Based on the Global Cancer Observatory (GLOBOCAN) data, in 2018 [1], there were over 18.1 million new breast cancer cases globally. It is the fourth-leading cause of death worldwide, with 626,679 (6.6%) fatalities, while it is the second-leading cause of death in the developing world.

Historically, Otto Warburg discovered that cancerous cells have different metabolic patterns compared to normal cells which contain a higher concentration of lactate than that of normal cells even in the presence of adequate oxygen. The high levels of lactate in the stroma of cancerous cells are caused by the high rate of glycolysis, and remain dependent on this process even though there is no lack of oxygen, a phenomenon known as the Warburg effect. Cancerous cells are dependent on lactate produced by these two cells to carry out both catabolic and anabolic metabolism. The high dependence of cancerous cells on the Warburg and the reverse Warburg effect has been proven in a clinical trial of the pyruvate kinase inhibitor bromopyruvate in hepatocellular carcinoma, where the inhibition of pyruvate metabolism into lactate resulted in a 98% reduction of tumor size. Changes in cancer metabolism are associated with reduced 5-year survival rates in breast cancer. This is assumed to be caused by a more acidic cancer microenvironment due to the high concentration of lactate in the extracellular fluid of cancer tissue. This acidic environment alters the charge of chemotherapeutic agents, thereby reducing their effectiveness.

The presence of Warburg and reverse Warburg effects is also associated with increased invasiveness of cancer cells due to changes in extracellular matrix conformation and increased activity of matrix metalloproteinases (MMPs) secreted by macrophages and cancer cells. The activity of Warburg and reverse Warburg effects on breast cancer is reflected by the

expression of two molecules, namely caveolin-1 and monocarboxylate transporter-4 (MCT-4). MCT-4 is a transmembrane transport protein that functions in the transport of lactate from the cytoplasm into the intercellular fluid. In triple-negative breast cancer (TNBC), high expression of MCT-4 correlates with poor prognosis. Until now, research related to MCT-4 has only been limited to oral cancer and TNBC.

Table 1: Relationship between MCT-4 expression and age in breast cancer

Fisher's exact test				Independent t-test				
Age	MCT-4 expression			MCT-4	n	Age (years)	Mean	p
(years)	High (%)	Low (%)	p				difference	
≥40	38 (61.3)	13 (21.0)	0.077	Low	19	49.32 ± 13.11	0.246	0.933
<40				High	43	49.07 ± 9.387		

MCT-4: Monocarboxylate transporter-4

Methods

This study is an analytical study using a cross-sectional method aiming to determine the relationship between MCT-4 expression with breast cancer clinicopathology and subtypes. This study was conducted from April to May 2020 in Sanglah Central Public Hospital, Denpasar. The number of samples was 62 people.

This study uses paraffin-embedded biopsy tissue samples obtained from the Anatomical Pathology Department of Sanglah Central Public Hospital, Denpasar. Patient-related data including age, histological grade, tumor stage, tumor size, lymph node involvement, or systemic metastases were recorded on a standardized data collection sheet. All collected samples were processed to evaluate the expression of MCT-4. Tissue samples were obtained from incisional or excisional biopsies and were embedded paraffin. The tissue samples were then processed and the MCT-4 expression profile was evaluated by standard immunohistochemical techniques.

The inclusion criteria in this study were female patients diagnosed with breast cancer at Sanglah General Hospital, Denpasar. Subjects with a poor general condition in which biopsy was not viable and patients who refused to be involved in the study were excluded. Data analysis was performed using SPSS 25 software for Windows.

Patients with breast cancer who met the inclusion criteria for this study underwent tumor biopsy. The tumor tissue was sent to the histopathology laboratory and preserved in paraffin blocks. The samples were grouped based on the subtype of breast cancer. The samples used were breast cancer biopsy samples of Luminal-A, Luminal-B, and HER2 subtypes. MCT-4 expression was evaluated using the standard avidin–biotin method using rabbit (H-90; Sc-50329 Santacruz Biotech was diluted in 1:250). Preparations were de-paraffined with xylene and then rehydrated with 100%, 95%, and 70%, respectively, with each duration of 2, 1, and 1 min and finally another 1 min with water. Antigen recovery was done in citrate buffer 10 Mm, pH 6.0 for 10 min in a pressure cooker. The preparation was then cooled in room temperature and washed using phosphate-buffered saline. Blocking with H2O2 3% was performed in 15 min continued by endogen biotin blocking using DakoCyomation Biotin Blocking System (#X0590). Preparation was incubated in goat serum 10% for 1 h, then followed by incubation at 4°C with primary antibody for 24 h. Interaction between primary antibody with antigen was detected by secondary biotinilized antibody (Vector Labs, #BA-1000), then followed by streptavidin-HRP (Dako #K1016). Immune reactivity was detected with Dako Liquid + Substrate-Chromogen System. Staining system was then classified into three groups: 0 = unstained, 1 = lightly stained and diffuse or strongly stained <30% stromal cell, and 2 = strongly stained >30% stromal cell. In this study, MCT-4 variable will be grouped to 2, high group (Score 2) and low group (Score 0–1).

Results

Relationship between MCT-4 expression and age in breast cancer

The analysis was carried out by classifying age into two categories (≥40 years and <40 years) based on age risk factors for breast cancer. The results of the analysis showed that the mean age of subjects with low MCT-4 expression was almost the same as the group of subjects with high MCT-4 expression. The mean age difference between the two groups was 0.246 years, which was not statistically significant. It can be seen that more subjects aged over 40 years old had high MCT-4 expression (38 patients, 61.3%) compared to patients below 40 years of age (8.1%). The proportion of MCT-4 expression in patients below 40 years of age was more balanced. However, the results of statistical analysis showed that the differences between the two age groups were not significant (Tables 1 and 2).

Bivariate analysis regarding the relationship between MCT-4 expression and clinicopathological characteristics in breast cancer patients

The relationship between MCT-4 expression and clinical staging in this study was analyzed using Chi-square analysis. The results of the analysis showed that MCT-4 was significantly associated with the clinical stage of breast cancer (Table 2). The proportion of advanced breast cancer patients with high MCT-4 expression was three times higher than the proportion of subjects with low MCT-4 expression in the same group. Conversely, in early-stage breast cancer

Table 2. Characteristics of the subjects in this study

Variable	Proportion (mean) (%)
Age (years)	49.15 ± 10.558
Stage	
I	2 (3.2)
II	17 (27.4)
III	34 (54.8)
IV	16 (25.8)
Histological grade	
I	3 (4.8)
II	17 (27.4)
III	42 (67.7)
Subtype	
Luminal A	15 (24.2)
Luminal B	25 (40.3)
HER2	13 (21.0)
TNBC	9 (14.5)
Histological type	
NST	44 (71.0)
DCIS	1 (1.6)
Invasive lobular	1 (1.6)
Invasive lobular	1 (1.6)
Invasive lobular	4 (6.5)
Invasive lobular	1 (1.6)
Pleomorphic type	
Mixed invasive	1 (1.6)
NST with lobular	
Mucinous	1 (1.6)
NOS	2 (3.2)
Papillary	1 (1.6)
Pleomorphic	4 (6.5)
Squamous cell	1 (1.6)
Ki-67	
Negative-low	16 (25.8)
High	46 (74.2)
MCT-4	
Low	19 (30.6)
High	43 (69.4)

MCT-4: Monocarboxylate transporter-4, TNBC: Triple-negative breast cancer, NST: No special type, NOS: Not otherwise specified, DCIS: Ductal carcinoma *in situ*, HER2: Human epidermal growth factor receptor 2

patients, there were more patients with lower MCT-4 expression. Fisher's exact analysis results showed that the difference between the two groups (early and advanced stages) was significant (p = 0.034).

Table 3: The relationship between MCT-4 expression and the stage, pathology, subtype, and histopahtological grade of breast cancer

Variable	MCT-4 expression		p
	High (%)	Low (%)	
Stage			
Early (I and II)	5 (41.7)	7 (58.3)	0.034
Advanced (III and IV)	38 (76.0)	12 (24.0)	
Pathology			
Invasive	30 (66.7)	15 (33.3)	0.455
Noninvasive	13 (76.5)	4 (23.5)	
Subtype			
Luminal	22 (55.0)	18 (45.0)	0.001
Nonluminal	21 (95.5)	1 (4.5)	
Histopatological grade			
High (Grade III)	34 (81.0)	5 (19.0)	0.004
Low (Grade I and II)	9 (45.0)	11 (55.0)	

MCT-4: Monocarboxylate transporter-4

Chi-square statistical test was also performed to analyze the relationship between MCT-4 expression and the pathology of breast cancer in this study. The proportion of subjects with invasive breast cancer was greater than that of non-invasive breast cancer. The results of the analysis showed that there was only a slight difference in pathological types between groups with high and low MCT-4 expression, where non-invasive tumors had a slightly higher tendency to have high MCT-4 expression compared to invasive tumors (76.5% vs. 66.7%). This difference had a p = 0.455, which is not statistically significant (Table 3).

Table 4. The ratio probability of the stage, subtype, and histopathological grade of breast cancer in high and low MCT-4 expression

Variable	MCT-4 expression		Adjusted-OR (95% CL)	p
	High (%)	Low (%)		
Stage				
Early (I and II)	5 (41.7)	7 (58.3)	4.727	0.047
Advanced (III and IV)	38 (76.0)	12 (24.0)	(1.019–21.922)	
Subtype				
Luminal	22 (55.0)	18 (45.0)	17.85	0.009
Nonluminal	21 (95.5)	1 (4.5)	(2.069–154.003)	
Histopatological grade				
High (Grade III)	34 (81.0)	5 (19.0)	1.957	0.327
Low (Grade I and II)	9 (45.0)	11 (55.0)	(0.512–7.489)	

MCT-4: Monocarboxylate transporter-4, OR: Odds ratio, CI: Confidence interval

The analysis of relationship between MCT-4 with histological grade was analyzed using the Chi-square test. To simplify the analysis and allow for risk analysis in the next stage, the histological grade variable was reclassified into low-grade (Grades I and II) and high-grade (Grade III) groups. Tabulation showed that high-grade tumors had a greater tendency to have a high MCT-4 expression (81%), while low-grade tumors had a more balanced proportion (high MCT-4: 45%; low MCT-4: 55%). The results of the analysis showed a significant p = 0.004, which indicates that MCT-4 tends to be overexpressed in tumors of high histological grade (Grade III).

The analysis of the association of MCT-4 with breast cancer subtypes is based on the fact that there are differences in the characteristics and levels of malignancy from one subtype to another. At this stage, the analysis was carried out by reclassifying the subtypes into luminal (Luminal A and B) and non-luminal (human epidermal growth factor receptor 2 [HER-2] and TNBC). The results of the analysis after re-classification showed that the non-luminal subtype had a much higher proportion of high MCT-4 expression compared to the luminal subtype (95.5% vs. 55.0% for non-luminal and luminal subtypes). The results of the analysis showed that the difference between the two analyses was significant (p < 0.05).

Multivariate analysis of the relationship between MCT-4 expression and clinicopathological characteristics in breast cancer

To evaluate the effect of each significant clinicopathological variable on MCT-4 expression independently, a multivariate analysis was performed. Logistic regression analysis was performed because both the dependent variable (MCT-4) and the analyzed covariates (stage, grade, and subtype) were nominal data types. The results of the analysis showed that of the three variables that were significantly associated with MCT-4 expression, only clinical staging and subtype (luminal and non-luminal) remained independently associated with MCT-4 expression. The results of the analysis on the clinical stage variable showed that the adjusted OR was 4.727 (p = 0.047; 95%CI: 1.109–21.922), while for the subtype variable, the adjusted OR was 17.850 (p = 0.009; 95%CI: 2.069–154.003) (Table 4). This shows that MCT-4 has a significant relationship with breast cancer clinical stage and subtype, and indicates an increased risk of cancer progression and more malignant subtypes (non-luminal).

Discussion

Based on the expression of MCT-4, our data showed that the majority of tumors expressed high levels of MCT-4. MCT-4 is a special lactate transporter that carries lactate from the intracellular to the extracellular environment to prevent the accumulation of lactate that is potentially toxic to cells (Dimmer *et al*., 2000) [2]. The role of MCT-4 is most crucial in rapidly dividing cells because these cells generally rely on oxidative glycolysis metabolism (Warburg effect), which leads to a sharp increase in lactate production in the intracellular environment (Franziska *et al*., 2015) [3]. The acidic nature of lactate may interfere with important cellular catalytic processes and cause apoptosis.

The bivariate analyses in this study showed that MCT-4 expression was associated with clinical stage, grade, and subtype. Age and pathological type showed no significant relationships with MCT-4 expression in this study. However, multivariate analysis showed that only two variables were independently associated with MCT-4 expression in breast cancer, namely clinical stage and subtype, which at the analysis stage were simplified into luminal and non-luminal. This is based on the significant differences in molecular aspects between the luminal and non-luminal subtypes and the tendency for higher levels of malignancy of the non-luminal subtypes (TCGA, 2012) [4]. However, the association of MCT-4 with Ki-67 was only significant at the bivariate analysis level and became insignificant at the multivariate analysis. This is probably due to the close relationship between Ki-67 and breast cancer subtypes, where more malignant subtypes tend to express higher levels of Ki-67 (Parker *et al*., 2009) [5]. Therefore, it appears that the type of subtype is an independent determinant of MCT-4 and not Ki-67.

Increased expression of MCT-4 is a crucial marker of metabolic change. Cancer cells that divide rapidly require a large supply of carbon skeletons that cause changes in the metabolic pattern of these cells (Tennant *et al*., 2010) [6]. This fact is reflected by the significant relationship between Ki-67 and MCT-4 expression in this study. Ki-67 is known to be a mitotic marker that is widely used to determine the rate of cell division in cancer (Parker *et al*., 2009) [5]. Cells in hypoxic areas of cancer mass produce large amounts of lactate generated by both oxidative glycolysis and anaerobic glycolysis. The lactate is used by cells in non-hypoxic areas as respiratory fuel and as an additional source of carbon skeletons, resulting in a continuous symbiosis between hypoxic and non-hypoxic cancer cells known as the reverse Warburg effect (Vander Heiden *et al*., 2009; Ward *et al*., 2012) [7], [8]. The high concentration of lactate in the cancer microenvironment accompanied by a decrease in glucose concentration due to the high rate of cancer glycolysis causes several pathogenic molecular effects as described below.

The existence of the Warburg and reverse Warburg effects benefits cancerous cells because it helps reduce the suppression of cancer growth by the anti-cancer immune system (cytotoxic T cells and NK cells). As previously discussed, the Warburg effect also causes the accumulation of lactate in the cancer microenvironment. Lactate causes the acidification of the cancer microenvironment that provides ideal conditions for the action of MMP enzymes, especially MMP-9 and MMP-12 (Han *et al*. 2013) [9]. Acidic conditions can also induce the increased expression of MMP as well as type 2 collagen, which can be used as a migratory pathway by invasive cancer cells. Therefore, the increased activity of Warburg effect in cancer is associated with an enhanced cancer progression, mainly due to an increase in the rate of proliferation and the invasiveness of the cancer mass. Furthermore, because of the close relationship between MCT-4 and these metabolic changes, conceptually, MCT-4 is a strong biomarker of cancer progression including breast cancer.

Baenke *et al*. (2015) [10], through functional screening analysis, showed that MCT-4 is an important survival regulator in breast cancer cells. This result was found to be consistent in 17 models of immortal cancer cells, and therefore, it can be deduced that the role of MCT-4 is not limited only to certain breast cancer subtypes, although different breast cancer subtypes may have gradations in their dependence on MCT-4. The above-said study showed that MCT-4 gene silencing may lead to an increased dependence of cancer cells on mitochondrial metabolism accompanied by a decrease in proliferation rate, MMP expression, and increased sensitivity to doxorubicin. Similar results were also found in model cells from other cancers such as bladder cancer and melanoma (Pinheiro *et al*., 2016; Todenhöfer *et al*., 2018) [11], [12]. Furthermore, decreased expression of MCT-4 in TNBC model cells induced a significant increase in NK cell functionality (Long *et al*., 2018) [13]. This was observed from the increased production of perforin in NK cells and the production of IFN-ɣ by NK cells which is a strong indicator of NK cell activation. This suggests that the expression level of MCT-4 may have an impact on the level of immunosurveillance and activation of the anti-cancer immune system which is known to contribute to prognosis.

Conclusion

We may conclude from this study that high MCT-4 expression is not significantly associated with younger age groups and the type of pathology in

breast cancer patients. High MCT-4 expression was significantly associated with advanced breast cancer (Stage III and IV), high histological grade (grade III), and malignant subtype (HER-2 and TNBC) in breast cancer patients.

Research Ethics

Ethical approval was obtained by the Ethics Committee, Faculty of Medicine, Udayana University, Sanglah General Hospital, Bali, Indonesia.

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