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Published by Intisari Sains Medis

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



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Received: 2021-11-28

Accepted: 2022-01-31

Published: 2022-02-07

ABSTRACT

Background: 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 transports lactate from the cytoplasm to the intercellular fluid.

Method: 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 the covariates (stage, grade, and subtype). Of the three variables 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, increasing the risk of cancer stage progression and developing a more malignant (non-luminal) subtype.

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

Keywords: Breast cancer, Monocarboxylate Transporter-4 (MCT-4).

Cite This Article: Nicolas, G.A., Sudarsa, I.W., Adiputra, P.A.T., Wihandani, D.M., Supadmanaba, I.G.P. 2022. Relationship between Monocarboxylate Transporter 4 (MCT-4) Expression and Breast Cancer Clinicopathology and Subtype in Sanglah General Hospital, Denpasar, Indonesia. *Intisari Sains Medis* 13(1): 30-34. DOI: [10.15562/ism.v13i1.1274](https://doi.org/10.15562/ism.v13i1.1274)

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, contributing approximately 11.6% of all new cases in 2018. Based on GLOBOCAN Global Cancer Observatory data, in 2018, 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.¹

Historically, Otto Warburg discovered

that cancerous cells have different metabolic patterns than normal cells. It contains a higher lactate concentration than normal cells, even inadequate oxygen. The high rate of glycolysis causes high lactate levels in the stroma of cancerous cells.² They 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.³ 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.⁴

Warburg and Reverse-Warburg effects are also associated with increased invasiveness of cancer cells due to changes

in extracellular matrix conformation and increased activity of MMPs secreted by macrophages and cancer cells.⁵ 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 transports lactate from the cytoplasm into the intercellular fluid.^{6,7} In triple-negative breast cancer, high expression of MCT-4 correlates with poor prognosis.⁸ Research related to MCT-4 has only been limited to oral cancer and triple-negative breast cancer. Therefore, it is important to investigate the MCT-4 expression globally in all types of breast cancer.

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 in paraffin. The tissue samples were then processed and the MCT-4 expression profile 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 rabbit's standard avidin-biotin method (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 minute and finally another one minute with water. Antigen recovery was done in citrate buffer 10 Mm, Ph 6.0 for 10 minutes in a pressure cooker. The preparation was then cooled to room temperature and washed using PBS. Blocking with H₂O₂ 3% was performed in 15 minutes, continued by endogen biotin blocking using DakoCyomation Biotin Blocking System (#X0590). Preparation was incubated in goat serum 10% for 1 hour, followed by incubation at 4 degrees Celcius with primary antibody for 24 hours. The interaction between primary antibody with antigen was detected by secondary biotinized antibody (Vector Labs, #BA-1000) followed by streptavidin-HRP (Dako #K1016). Immune reactivity was detected with Dako Liquid + Substrate-Chromogen System. Staining system were then classified into three groups: 0 = unstained, 1 = lightly stained and diffuse or strongly stained <30% stromal cell, 2 = strongly stained >30% stromal cell. In this study, MCT-4 variable will be grouped into 2, high group (score 2) and low group (score 0-1).

RESULTS

Relationship between MCT-4 expression and age in breast cancer

Baseline characteristics of the study subjects are presented in Table 1. 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 analysis results showed that subjects with low MCT-4 expression mean age 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 statistical analysis results showed that the differences between the two age groups were not significant (Table 3).

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 analysis results showed that MCT-4 was significantly associated with the clinical stage of breast cancer. The proportion of the advanced breast cancer patients with high MCT-4 expression was three times higher than that of subjects with low MCT-4 expression in the same group. Conversely, there were more patients with lower MCT-4 expression in early-stage breast cancer patients. Fisher's exact analysis results showed that the difference between the two groups (early and advanced stages) was significant ($p=0.034$) (Table 2).

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 analysis results showed 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 than invasive tumors (76.5% vs. 66.7%). This difference had a p-value of 0.455, which is not statistically significant.

The relationship between MCT-4 with histological grade was analyzed using the chi-square test. The histological grade variable was reclassified into a low grade (grades I and II) and high-grade (grade III) groups to simplify the analysis and allow for risk analysis in the next stage.

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 analysis results showed a significant p-value of 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. The analysis was carried out at this stage by reclassifying the subtypes into luminal (Luminal A and B) and non-luminal (HER-2 and TNBC). After re-classification, the analysis results showed that the non-luminal subtype had a much higher proportion of high MCT-4 expression than the luminal subtype (95.5% vs. 55.0% for non-luminal and luminal subtypes). The analysis results showed that the difference in 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 the dependent variable (MCT-4) and the analyzed covariates (stage, grade, and subtype) were nominal data types. The analysis results 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 analysis results 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). 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).

Table 1. Subject's baseline characteristics.

Variable	Proportion (Mean)
Age	49.15 ± 10.558 years
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%)

DISCUSSION

Based on the expression of MCT-4, our data showed that most 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.⁸ 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.⁹ 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 malignancy of the non-luminal subtypes.⁹ However, 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, while more malignant subtypes tend to express higher levels of Ki-67. Therefore, it appears that

the type of subtype is an independent determinant of MCT-4 and not Ki-67.^{10,11}

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. This fact is reflected by the significant relationship between Ki-67 and MCT-4 expression in this study. Ki-67 is a mitotic marker that is widely used to determine the rate of cell division in cancer.^{12,13} Cells in hypoxic areas of cancer mass produce large amounts of lactate generated

by 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. 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.^{14,15}

The Warburg and Reverse Warburg effects benefit 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 lactate accumulation 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-12.¹⁶ Acidic conditions can also induce the increased expression of MMP and type 2 collagen, which can be used as a migratory pathway by invasive cancer cells. Therefore, the increased activity of the 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

Table 2. Bivariate analysis of MCT-2 expression according to the stage, pathology, subtype, and grade.

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

Table 3. The bivariate analysis of MCT-4 expression according to subject's age.

Age	Fisher's Exact Test			Independent t-test				
	MCT-4 Expression		p	MCT-4	N	Age (years)	Mean difference	p
	High	Low						
≥40 years	38 (61.3%)	13 (21.0%)	0.077	Low	19	49.32 ± 13.11	0.246	0.933
< 40 years	5 (8.1%)	6 (9.7%)		High	43	49.07 ± 9.387		

Table 4. Multivariate analysis of MCT-4 expression according to the stage, subtype, and grade.

Variable	MCT-4 Expression		Adjusted-OR (95 % CI)	p-value
	High	Low		
Stage			4.727 (1.019-21.922)	0.047
Early (I and II)	5 (41.7%)	7 (58.3%)		
Advance (III and IV)	39 (76.0%)	12 (24.0%)		
Subtype			17.85 (2.069-154.003)	0.009
Luminal	22 (55.0%)	18 (45.0%)		
Non-Luminal	21 (95.5%)	1 (4.5%)		
Histopathological Grade			1.957 (0.512-7.489)	0.327
High (Grade III)	34 (81.0%)	5 (19.0%)		
Low (Grade I and II)	9 (45.0%)	11 (55.0%)		

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.¹⁷

Several studies showed that MCT-4 is an important survival regulator in breast cancer cells.¹⁸ This result was consistent in 17 models of immortal cancer cells. Therefore, it can be deduced that the role of MCT-4 is not limited only to certain breast cancer subtypes. However, different breast cancer subtypes may have gradations in their dependence on MCT-4. Said study showed that MCT-4 gene silencing might 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.^{19,20}

Furthermore, decreased MCT-4 in TNBC model cells increased NK cell functionality significantly. This was observed from the increased production of perforin in NK cells and the production of IFN- γ by NK cells which is a strong indicator of NK cell activation. This suggests that the expression level of MCT-4 may impact the level of immunosurveillance and activation of the anti-cancer immune system, which is known to contribute to prognosis.²¹

CONCLUSION

We may conclude from this study that high MCT-4 expression is not significantly associated with the younger age group 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.

CONFLICTS OF INTEREST

There is no conflict of interest in writing this research report.

RESEARCH ETHICS

Ethical approval was obtained by the ethics committee, Faculty of Medicine, Udayana University, Sanglah General Hospital, Bali, Indonesia, with ethical clearance number: 1562/UN14.2.2.VII.14/LT/2019.

FUNDING

The author is solely responsible for funding this research without sponsors or other sources.

AUTHOR CONTRIBUTION

All authors contributed equally in the writing of this article.

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