RESEARCH ARTICLE

INDONESIAN JOURNAL OF CANCER

High CD90 Expression is A Predictor of Axillary Nodal Metastasis in Breast Carcinoma

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ARTICLE INFO

Received : 15 July 2023
Reviewed : 19 July 2023
Accepted : 22 August 2023

Keywords:
breast carcinoma, cancer stem cell, CD99

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ABSTRACT

Background: Metastasis is the most common cause of death in breast cancer and nodal metastasis is one of the most important predictive factors for distance metastasis. One of the intrinsic properties of cancer cells is that they resemble stem cells which are capable of self-renewal, resistance to chemotherapy and radiation, and metastasis. CD90 is a stem cell marker found in several malignancies, including breast carcinoma. The objective of this study was to prove that CD90 can predict the occurrence of axillary nodal metastasis in breast carcinoma.

Methods: The study design was a case-control study. The subjects of this study were breast cancer patients at Prof. Dr. I. G. N. G. Ngoerah Hospital who underwent mastectomy and axillary lymphadenectomy in the year 2019. CD90 immunohistochemistry was performed and its association with metastasis, along with various clinicopathological markers, was assessed with chi-square and logistic regression tests with a significance level determined at α=0.05.

Results: There were 25 cases of breast carcinoma with axillary nodal metastasis and 25 cases without metastasis. There was a significant relationship between high CD90 expression and the occurrence of nodal metastasis (p=0.010). There was no relationship between patient age, histologic grade, histologic subtype, molecular subtype, and T-stage with axillary nodal metastasis. Breast carcinoma patients with high CD90 expression have a 7.25 times higher nodal metastasis risk compared to patients with low CD90 expression.

Conclusion: High CD90 expression could predict axillary nodal metastasis in breast carcinoma.

INTRODUCTION

Breast cancer is the most common malignancy in women worldwide, and its incidence is increasing [1–3]. In Indonesia, due to the lack of breast cancer screening availability and awareness, as well as social and economic limitations and low level of education, most cases of breast cancer come at an advanced stage with a high mortality rate. Most (>90%) of deaths in breast cancer patients are due to distant metastasis. Metastasis that are often found in breast cancer are metastases to bones, lungs, liver, and brain [4]. Available prognostic biomarkers for breast cancer are ER, PR, HER-2, Ki-67, E-Cadherin, circRNAs, p53, miRNAs, Tumor-Associated Macrophages (TAMs), and Inflammation-Bases Models [5]. Until now, nodal metastasis is the most important predictor for distance metastasis, meanwhile, not all breast cancer patients are indicated to mastectomy with axillary lymph node dissection [6]. Many biomarkers are still in research, including stem cell markers. Cancer stem cells (CSCs) are cancer cells with the ability to self-renewal and differentiate, resist chemotherapy and radiation, and initiate new tumors in a distant site [7].

Many CSCs markers in breast cancer have been studied extensively, such as CD90, CD44, EpCAM, ALDH1, SOX1, etc [8]. CD90 or Thy-1 is a membrane glycophosphatidyl inositol anchored protein with a molecular weight of 25–37 kDa. Messenger RNA expression and CD90 protein expression have been reported in several malignancies, including breast carcinoma. Some of the roles of CD90 in cancer include as a marker of CSCs, as a prognostic marker, as well as regulating migration and metastasis. Metastasis is a complex process involving multiple properties of cancer cells and the interaction of cancer cells with the tumor microenvironment. Epithelial-mesenchymal transition

(EMT) is an important biological changes in cancer that facilitate metastasis. EMT program up-regulates CD90 in cancer cells which mediates the physical interaction between CSCs with TAMs [9]. This study aimed to determine that high CD90 expression can be used to predict the occurrence of axillary nodal metastasis in breast carcinoma.

METHODS

Patient

This study was a case-control study using 25 breast cancer patients with axillary node metastasis as a case group and 25 patients without metastasis as a control group. The subject was a breast cancer patient who underwent biopsy, mastectomy, and radical axillary dissection and the histopathological examination was performed at the Anatomical Pathology Laboratory of Prof. Dr. I. G. N. G Ngoerah Hospital from 1 January 2019 to 31 December 2019. Patient age data was collected from medical records. Histological type, molecular subtype, and histological grade data were collected from the histopathological report. Tumor size (T stage) and nodal status were collected from histopathological reports, except in patients with mastectomy post neoadjuvant chemotherapy, data was retrieved from clinical data.

Immunohistochemistry

The immunohistochemistry was done in a tumor biopsy specimen before chemotherapy using monoclonal anti-CD90 antibody ab133390 (Abcam, UK) with 1:50 dilution. Heat antigen retrieval was done with citrate buffer at pH 6, at 1000C, for 15 minutes.

Interpretation of CD90 expression was using an Olympus CX22 binocular light microscope. CD90 expression was asses semiquantitatively using the H-score method based on multiplication of the percentage of stained cells and the intensity of the staining. Staining intensity was cathagorized into 0 (negative), 1 (low), 2 (moderate), and 3 (strong) **(Figure 1).** CD90 expression was categorized into high and low expressions with the median value as the cut-off point.

Statistical analysis

Data was tested using SPSS (Statistical Package for the Social Sciences) 25.0 for Windows. All clinicopathological characteristics and CD90 expression were assessed for their association with the occurrence of metastases in breast carcinoma with bivariate analysis by the chi-square test and multivariate analysis by logistic regression test. The significance level was determined at α=0.05.

RESULTS

The subjects of this study were 25 breast cancer patients with axillary nodal metastasis at Prof. Dr. I. G. N. G Ngoerah hospital who were selected consecutively for the case group and 25 breast carcinoma patients without axillary nodal metastasis for the control group. The age range of the patients was 35–75 years old with an average of 53.28 years old. Characteristics of breast carcinoma patients based on age, histological grade, histological and molecular subtypes, and T stage are presented in **Table 1**.

All clinicopathological characteristics and CD90 expression were assessed for their association with the occurrence of axillary nodal metastasis with bivariate analysis by chi-square test (**Table 2**).

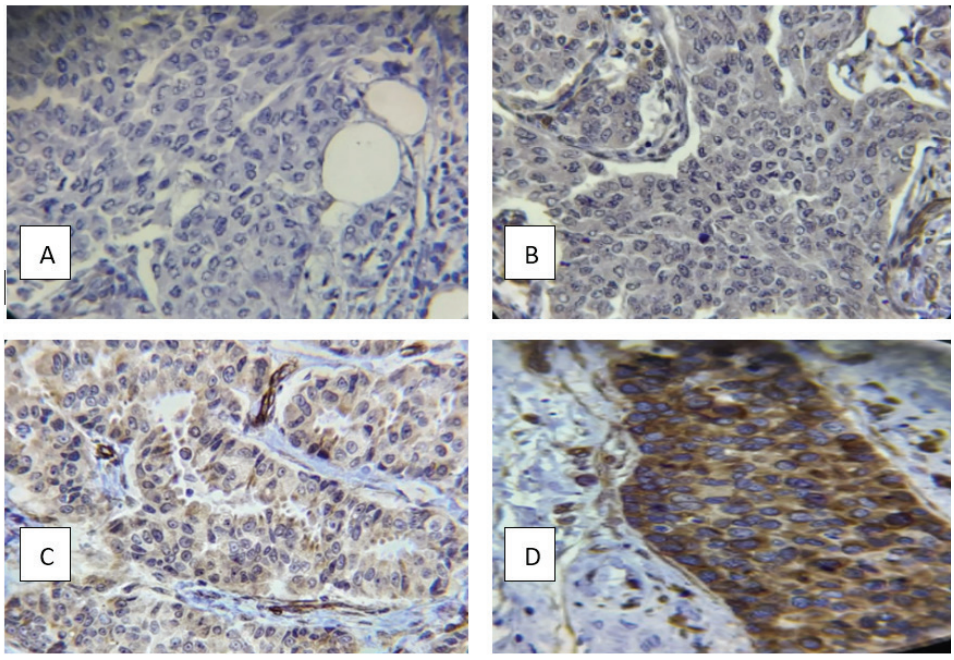

Figure 1. CD90 expression in breast carcinoma tumor cells with varying staining intensity:
(A) Negative;
(B) Weak intensity;
(C) Moderate intensity;
(D) Strong intensity. (CD90 munohistochemistry, 400x)

Table 1. Distribution of breast carcinoma patients based on clinic-pathological characteristics.

Characteristics	n	Percentage
Age (year old)		
<50	21	42%
≥50	29	58%
Histological grade		
1–2	36	72%
3	14	28%
Histological subtype		
IC NST	36	72%
Others	14	28%
Molecular subtype		
Luminal A	11	22%
Luminal B	29	58%
HER2-enriched	5	10%
TNBC	5	10%
T stage		
T1–T2	25	50%
T3–T4	25	50%
CD90 expression		
Low	21	42%
High	29	58%

IC NST: Invasive Carcinoma of No Special Type; HER2: Human Epidermal Growth Factor Receptor 2; TNBC: Triple Negative Breast Carcinoma.

Multivariate analysis was also performed to assess the correlation between clinicopathological characteristics and CD90 expression with the occurrence of axillary node metastasis. The result revealed that only CD90 expression had a significant correlation with axillary nodal metastasis (p=0.010), with an odd ratio (OR) was 7.25 (95% confidence interval 1.61–32.67) (**Table 3**).

Based on the results of bivariate and multivariate analysis, there was a significant association between CD90 expression and axillary node metastasis. Breast carcinoma patients with high CD90 expression had a 7.25x higher risk of developing nodal metastases when compared to breast carcinoma patients with low CD90 expression.

DISCUSSION

Nodal metastasis is the most important predictor for distance metastasis and nodal status is important in decision-making whether a patient will treated with chemotherapy [6]. Many studies have been done to find tools to predict nodal metastasis, such as imaging studies, clinicopathological factors, and molecular studies. Clinicopathological factors that are significantly related to nodal metastasis are lymphovascular invasion (LVI) status and tumor size [10]. In this study, tumor size (T stage) has no significant association with nodal status.

Our knowledge about the acquired capability of cancer cells which is important for tumor growth is evolving. Hanahan & Weinberg [11] have begun to introduce 6 hallmarks of cancer which were self-sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion and metastasis, limitless replicative potential, sustained angiogenesis, and evading apoptosis. In 2011, 4 new hallmarks were described which were avoiding immune destruction, tumor-promoting inflammation, genome instability and mutation, and deregulating cellular energetics. In 2022, another 4 emerging hallmarks were added which were unlocking phenotypic plasticity, non-mutational epigenetic reprogramming, polymorphic microbiome, and senescence cells [12].

Metastasis is a complex process, starting from the penetration of malignant cells outside the basement membrane into the surrounding stroma, invasion of malignant cells into the blood vessels and then joining the circulation, penetration of malignant cells outside the blood vessels, until finally, they grow into a new tumor mass in the same place away from the primary tumor. At the initial stage of invasion, cancer cells undergo a functional change from cells that have an epithelial shape to cells that are spindle-shaped, loosely arranged, and more motile. This change is called the epithelial-mesenchymal transition (EMT). Meanwhile, at the new implantation site, the carcinoma cells will return to their original epithelial cell type. This change is called the mesenchymal-epithelial transition (MET) [13].

Cancer cells are formed by clonal expansion of a single genetically damaged precursor cell. Various genetic and epigenetic changes then give rise to heterogeneous cancer cells, which have various abilities that allow tumors to continuously develop, invade, and metastasize. One of the properties of these heterogeneous tumor cells is that the cells have stem cell-like properties [14]. These stem cells will be a source of new cancer cells, have properties resistant to chemotherapy and radiation, as well as a source of metastases. A review by Hanahan [12], summarized 3 ways in which cancer cells obtain phenotypic plasticity and can stay in a progenitor-like state, they are 1) dedifferentiation from mature cells to progenitor cells; 2) blocked differentiation from progenitor state; and 3) trans-differentiation from another mature type cells.

CD90 is one of the stem cell markers that has been studied in many malignancies. CD90 or Thy-1 is a membrane glycophosphatidylinositol anchored protein with a molecular weight of 25-37 kDa. mRNA expression and CD90 protein expression have been reported in several malignancies, including breast carcinoma. Some of the roles of CD90 in cancer include as a marker of cancer stem cells (CSC), as a prognostic marker, as well as regulating migration and metastasis. The last point

Table 2.
Correlation between clinicopathological characteristics and CD90 expression with the occurrence of nodal metastases in breast carcinoma tested with bivariate analysis

Characteristics	Metastasis		n	OR	95% CI	*P**
	Negative (n=25)	Positive (n=25)				
Age (year old)						
<50	10	11	21			
≥50	15	14	29	0.85	0.78–2.62	0.774
Histological grade						
1–2	19	17	36			
3	6	8	14	1.49	0.43–5.17	0.529
Histological subtype						
IC NST	18	18	36			
Others	7	7	14	1.00	0.29–3.44	1.000
Molecular subtype						
Luminal A	6	5	11			
Luminal B	12	17	29			
HER2-enriched	4	1	5			
TNBC	3	2	5	**		0.399
Tumor size						
T1–T2	15	10	25			
T3–T4	10	15	25	0.34	0.49–8.91	0.157
CD90 expression						
Low	15	6	21			
High	10	19	29	4.75	1.41–16.06	0.010

*significant if p <0.05
**Risk estimation statistics cannot be computed. They are only computed for 2x2 table without empty cells
IC NST: Invasive Carcinoma of No Special Type; HER2: Human Epidermal Growth Factor Receptor 2; TNBC: Triple Negative Breast Carcinoma

Table 3.
Correlation between clinicopathological characteristics and CD90 expression with the occurrence of nodal metastases in breast carcinoma tested with multivariate analysis

Characteristics	Metastasis		n	OR	95% CI	*P**
	Negative (n=25)	Positive (n=25)				
Age (year old)						
<50	10	11	21	2.59	0.53–12.6	0.24
≥50	15	14	29			
Histological grade						
1–2	19	17	36			
3	6	8	14	3.55	0.63–4.62	0.15
Histological subtype						
IC NST	18	18	36	0.85	0.16–6.62	0.85
Others	7	7	14			
Molecular subtype						
Luminal A	6	5	11			
Luminal B	12	17	29	1.23	0.21–7.31	0.82
HER2-enriched	4	1	5	0.10	0.00–2.41	0.16
TNBC	3	2	5	0.12	0.01–2.74	0.19
Tumor size						
T1–T2	15	10	25			
T3–T4	10	15	25	2.09	0.49–8.91	0.32
CD90 expression						
Low	15	6	21			
High	10	19	29	7.25	1.61–32.67	0.01

*significant if p <0.05
IC NST: Invasive Carcinoma of No Special Type; HER2: Human Epidermal Growth Factor Receptor 2; TNBC: Triple Negative Breast Carcinoma

is important, considering that more than 90% of cancer deaths are due to metastases [15,16].

Cancer stem cells began to be proposed four decades ago. It is thought that tumor development is determined by specific cell subsets, which are characterized by self-renewal properties, are multipotent, and can initiate tumors. Recently, the role of CD90 has been extensively studied in relation to CSC. The ability to form tumors in vivo in immune-deficient mice is thought to be the most important property of CSCs [15].

The prognostic role of CD90 depends on the type of cancer. In glioblastoma, high CD90 expression in tumor specimens correlates with the invasive nature of the tumor as demonstrated by imaging techniques. These imaging features are associated with shorter patient survival. Therefore, CD90 expression was used to stratify patients who could be treated with dasatinib. In hepatoblastoma, increased CD90 expression is significantly associated with advanced disease, poor response to therapy, and lower survival. CD90 overexpression has also been identified as a poor prognostic marker in acute myeloid leukemia. In contrast, CD90 also exhibits tumor suppressor function in some malignancies. CD90 downregulation is associated with poor prognosis and disease progression in ovarian carcinoma, neuroblastoma, and nasopharyngeal carcinoma. Overall, the above suggests an ambivalence of CD90 function, with pro- or anti-tumor properties depending on tumor type [15].

Tumor invasion and tumor migration are hallmarks of cancer underlying tumor dissemination. Recent studies have demonstrated the invasive and metastatic capacity of cancer cells expressing CD90 in several malignancies. These cancer cells have the capacity to invade surrounding tissues, form spheroids in vitro, and show high expression of TWIST1 and TWIST2, which are two important transcription factors involved in the epithelial-mesenchymal transition (EMT) process [15].

In an in vitro study using normal breast cancer cell lines, CD90 was more expressed by cancer cell lines than the 9 CSC markers studied, while CD14 was expressed more by non-tumorigenic cell lines [17].

A study by Lobba et al. [16] in breast cancer patients found that CD90 positivity correlated with metastases and poor patient survival in patients with the basal-like subtype. Using a functional genetic approach to normal and malignant breast cell lines shows that CD90 is involved in several cellular processes such as changes in cell shape, increased cell proliferation, invasive and metastatic capabilities, and activation of the EGFR pathway. This protein also appears to be a candidate for new targeted therapy, particularly in basal-like subtype breast cancer [18].

In a study by Yamashita & Kaneko [19] in hepatocellular carcinoma, c-KIT was found to be upregulated in CD90+ cells isolated in patient samples. Therefore treatment with imatinib may lead to the reduction in the metastatic potential of the CD90+ cells.

In this study, high CD90 expression was significantly associated with the occurrence of nodal metastases in breast cancer patients. Patients with breast carcinoma with high CD90 expression have a 7.25 times higher risk of developing nodal metastases when compared to breast carcinoma with low CD90 expression. The strength of this study is that CD90 is a CSC marker and EMT and it can be assessed with immunohistochemistry which is a relatively simple procedure. Due to the limited sample size, large-scale and multicentric studies are needed.

CONCLUSIONS

There was a significant association between metastasis and CD90 expression. Breast carcinoma patients with high CD90 expression had a 7.25 times higher risk of developing nodal metastases when compared to breast carcinoma patients with low CD90 expression. Evaluation of stem cell expression in cancer patients can predict the likelihood of recurrence, metastasis, poor survival, drug resistance, and specific therapeutic targets.

Large-scale and multi-centric studies are needed to prove the consistency of this study's results. CD90 is an important candidate for targeted therapy in breast cancer. Research in this area is important.

DECLARATIONS

Competing interest

The authors declare no competing interest in this study.

Ethical approval

This study obtained ethical approval from the Research Ethical Commission of the Faculty of Medicine, Udayana University with ethical approval number: 701/ UN14.2.2.VII.14/LT/2020.

Funding

This study was funded by PNBP research grant from Udayana University with assignment agreement number: B/20-133/UN14.4.A/PT.01.05/2020.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49.
2. Paskarani P, Sriwidyani N, Kurniasari N, et al. The Burden of Cancer in Bali: An Epidemiology Report 2017-2019. Int J Med Rev Case Reports. 2022;6(0):1.
3. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin. 2023;73(1):17–48.

4. Medeiros B, Allan AL. Molecular mechanisms of breast cancer metastasis to the lung: Clinical and experimental perspectives. Int J Mol Sci. 2019;20(9).
5. Stanisławek A. Breast Cancer-Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies- An Updated Review. 2021;1–30.
6. Ali B, Mubarik F, Zahid N, Sattar AK. Clinicopathologic Features Predictive of Distant Metastasis in Patients Diagnosed With Invasive Breast Cancer. JCO Glob Oncol. 2020;(6):1346–51.
7. Qiu Y, Wang L, Zhong X, et al. A multiple breast cancer stem cell model to predict recurrence of T1-3, N0 breast cancer. BMC Cancer. 2019;19(1):729.
8. Shan NL, Shin Y, Yang G, et al. Breast cancer stem cells: A review of their characteristics and the agents that affect them. Mol Carcinog. 2021 Feb;60(2):73-100.
9. Lu H, Clauser KR, Tam WL, et al. A breast cancer stem cell niche supported by juxtacrine signalling from monocytes and macrophages. Nat Cell Biol. 2014 Nov;16(11):1105-17.
10. Yoshihara E, Smeets A, Laenen A, et al. Predictors of axillary lymph node metastases in early breast cancer and their applicability in clinical practice. Breast. 2013;22(3):357–61.
11. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell. 2011;144(5):646–74.
12. Hanahan D. Hallmarks of Cancer: New Dimensions. Cancer Discov. 2022;12(1):31–46.
13. Smigiel JM, Taylor SE, Bryson BL, et al. Cellular plasticity and metastasis in breast cancer: a pre- and post-malignant problem. J Cancer Metastasis Treat. 2019;2019 (June).
14. Kumar V, Abbas AK, Aster JC. Robbins Basic Pathology. 10th ed. Philadelphia: Elservier; 2018.
15. Sauzay C, Voutetakis K, Chatziioannou A, et al. CD90/ Thy-1, a Cancer-Associated Cell Surface Signaling Molecule. Front Cell Dev Biol. 2019 Apr 26;7:66.
16. Fico F, Bousquenaud M, Rüegg C, Santamaria-Martínez A. Breast Cancer Stem Cells with Tumor- versus Metastasis-Initiating Capacities Are Modulated by TGFBR1 Inhibition. Stem Cell Reports. 2019;13(1):1–9.
17. Maia Lobba AR, Oliveira Carreira AC, Dutra Cerqueira OL, et al. High CD90 (THY-1) expression positively correlates with cell transformation and worse prognosis in basal-like breast cancer tumors. PLoS One. 2018;13(6):1–21.
18. Redig AJ, McAllister SS. Breast cancer as a systemic disease: a view of metastasis. J Intern Med. 2013 Aug;274(2):113-26.
19. Yamashita T, Kaneko S. Liver cancer stem cells: Recent progress in basic and clinical research. Regen Ther. 2021;17:34–7.