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Association of SDF-1 with metastasis in breast cancer patient at Sanglah hospital, Bali-Indonesia

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ABSTRACT

Background: More than 24% breast cancer patients came to Sanglah Teaching Hospital with distant metastasis which cause 90% of cancer related death. Distant metastasis is complex process of interaction between tumor cells and its micro environment involving chemo-attractant cytokines which lead circulating tumor cells toward target organs. One of the most common cytokines involved in metastasis of multiple tumor is SDF-1, produces by target organ or tumor cells it selves. However, only few studies ever evaluate the relationship between its concentrations in tumor tissue with metastasis.

Method: A cross sectional analysis study was conducted involving clinical data and paraffin blocks from 46 patients. Samples were grouped into metastasis and non-metastasis group and level of tumor tissue SDF-1 was evaluated by immunohistochemistry method. Numerical conversion was done using modified "Mirisola" technique and statistical analysis was conducted using SPSS 16 software.

Results: The overall median expression of SDF-1 was 4in which the median is 3 in non-metastatic group and 6 in metastatic group. Elevated SDF-1 expression was significantly associated with increase metastatic risk (PR: 1.34; P=0.04; CI 95%: 1.014-1.769). In addition, parenchymal carcinoma cell had significantly higher expression of SDF-1 compared with micro-environmental cell (median SDF-1 in carcinoma vs microenvironment; 3vs.2; p=0,004). Finally, multivariateanalysis of SDF-1 expression also gave significant result that MBC had significantly higher expression of SDF-1 (p=0.019).

Conclusions: Elevated SDF-1 expressions significantly increase metastatic risk and majority of SDF-1 was produce by tumor parenchyma.

Keywords: Breast Cancer, SDF-1, Metastasis

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INTRODUCTION

Breast cancer is the second most prevalent cancer among women worldwide. In United States, the incidence is about 246.660 new cases in 2016, comprising 14.6% of all cancer incidences. It also one of leading cause of death among women in United States with 40.450 deaths in 2016. Breast cancer is also the most common cancer in Europe in 2006[1-4]. In Indonesia, breast cancer is the second highest malignancy in women after cervical carcinoma with tendency to increase in number in the upcoming years[5]. Sanglah General Hospital itself found new cases of breast cancer approximately 90 cases each year. More than 43% of patients with breast cancer came to Sanglah in advanced stage in which 26% had metastasis.[6]

The most feared consequences of malignancy are metastasis as its cause majority of cancer related deaths in all cancer types. In case of breast cancer, 90% of deaths are attributed to metastasis. The mechanism of metastasis is already extensively studied. However, there are still lots of elusive mechanism yet uncovered and new findings arise almost every year. One of new theoretical advancement in metastatic process is interaction between tumour parenchyma and tumour microenvironment (TME). This interaction is proved vital as microenvironment plays essential role to determine metastatic location and tumour response to therapy as well as length of survival.[7,8,9,10]

CXCL-12 (Chemokine Motif Ligand-12) or Stromal Cell-Derived Factor 1α (SDF-1) is a cytokine that produced by Carcinoma Associated Fibroblasts (CAFs). CXCL-12 is a specific ligand of (Chemokine Motif Receptor 4) CXCR-4 and plays role in the function of organogenesis, regeneration and tumorigenesis. They drive cells expressing CXCR4 close to cell expressing SDF-1.[11,12,13] The high expression of CXCR4 on breast cancer relates to younger age, larger tumour and lower rates of overalls suvival.[14] Expressions of both of these proteins are strongly influenced by HIF-1α which is mostly expressed by the hypoxic tissue.[15] In tumour tissue, hypoxic state begin when tumour mass reach 2 mm^3 in volume and 1% O_2 concentration, indicating that metastatic potential begin very early in tumour progression.[16,17]

The aim of this study is to evaluate the association between level of tissue SDF-1 and distant metastatic event. Currently, there is no similar research being conducted in Indonesia.

MATERIAL AND METHODS

The study was conducted at the Department of Surgery Medical Faculty of Udayana University/ Sanglah General Hospital. This cross sectional study was aimed to determine the relationship of the expression of SDF-1 in tumour tissue with distant metastases. Samples were taken from all paraffin blocks of breast cancer patients who had biopsy in 2009 to 2011 as well as clinical data from the patient's medical record. Research data collection began in July 2010. The study was conducted until sufficient sample. The inclusion criteria are biopsy sample of breast cancer patient treated in Sanglah general Hospital. All stages and types of breast cancer are included. Samples with incomplete clinical and pathological data, unrepresentative paraffin blocks, diagnosed other than breast cancer, or if the result of staining could not be interpreted were excluded.

The collected data included basic, clinical and pathological data. Data were divided into 2 groups: metastatic group and non-metastatic group. All samples with complete medical data were examined by using Immunohistochemistry (IHC) method to delineate CXCL12 / SDF-1 expression which were carried out in Laboratory of Pathology Anatomy Medical Faculty of Udayana University/ Sanglah General Hospital. We used rabbit monoclonal anti-SDF-1 antibody (AbCAM) diluted until 1:75. The data were analysed statistically by both bivariate and multivariate analysis. All analysis process was conducted using SPSS 16 for windows software.

Table 1 **Baseline characteristic of subjects**

Variables	No Metastases (n = 25)	Metastases (n = 21)	P
Ethnic			
Bali	17	12	0.97
Java	2	7	
Sasak	6	2	
Age			
Mean	48.20	43.24	0,054
SD	± 8.5	± 8.4	
Menstruation			
Premenopausal	18	19	0.11
Menopause	7	2	
Size			
Mean	11.4	8.3	0.80
SD	± 4.0	± 3.7	
Location			
Right	11	10	0.81
Left	14	11	
Histopathology			
Pathology results			
IDC	21	18	1.0
ILC	2	2	
Medullary	1	1	
Mucinous	1	0	
Tubular	0	0	
Micro Invasion			
Negative Invasion	14	18	0.03
Positive Invasion	11	3	
Mitosis			
Mean	21.48	15.57	0.08
SD	± 12.44	± 9.43	
TIL			
TIL Mild	8	14	0.13
TIL Moderate	11	4	
TIL Severe	6	3	

RESULTS

All samples were obtained from 2009-2011 register. 247 samples were collected in which 63 metastatic and 184 non-metastatic cases were collected during the courses of the study. However, only 89 samples had complete medical data. From these samples, 36 samples were classified as metastasis and 53 were non-metastasis. Only 46 samples were eligible for IHC-stained (21 patients with metastasis and 25 patients without metastasis). Of all samples, 37 samples were *Balinese*, 5 patients were *Sasak* tribe, and 4 patients were *Javanese*. The mean age of patients was 45.93 with the youngest was 30 years old and the eldest was 62 years. In term of menstrual status, 37 patients (80.4%) were on premenopausal age and 9 patients (19.6%) were already menopause.

The data about cancer grade and apoptotic were not obtained in this study because some samples were diagnosed other than IDC and apoptotic assessment was not routinely conducted in Laboratory of Pathology Anatomy Medical Faculty of Udayana University/ Sanglah General Hospital. Samples baseline characteristics are described in Table 1.

The result of IHC staining showed that the median value of SDF-1 expression was 4 from all samples. Separating the samples into metastatic and non-metastatic group reveal that metastatic group had higher level of SDF-1 compare to non-metastatic group (mean SDF-1 expression score 6 vs. 3). Logistic regression analysis showed that SDF-1 significantly increased metastatic risk (PR: 1.34; P=0.04; CI 95%:1.014-1.769) (Table 2). Moreover,

Table 2 Logistic regression analysis of association between SDF-1 expression and metastasis

					95.0% C.I. for OR	
Var	B	S.E	P-Value	PR	Lower	Upper
Sdf1	0.292	0.142	0.039	1.34	1.014	1.769

Table 3 Comparison of SDF-1 expression between carcinoma cell and tumour microenvironment

	Cell Type		
SDF-1 Expression	Carcinoma cell	*Microenvironment*	P
Median	3 (1-9)	2 (1-9)	0.001

Table 4 Multivariate analysis between several predictors with metastasis

				95.0% CI	
Predictor	B	P	Exp (B)	Lower	Upper
Age	-0241	0.016	0786	0647	0955
Size	0483	0.080	1,622	0944	2,786
Mitosis	-0266	0.007	0766	0631	0.930
TIL	-1471	0.442	0.230	0005	9,770
Invasion	3,279	0.057	26 537	0906	777 524
SDF1	0831	0.019	2,295	1,144	4602

cancer cells itself appear to produce more SDF-1 compared with micro-environmental cell in both metastatic and non-metastatic groups (Table 3). However, we found no correlation between SDF-1 and mitotic index in this study (Table 4).

Multivariate analysis of the relationship between the occurrences of metastases as dependent variables with several independent variables using logistic regression analysis showed that SDF-1 was significantly associated with metastasis. Other factors that showed similar result were age and mitosis. Table 4 summarize the result of multivariate analysis.

DISCUSSION

The relationship between SDF-1 and metastasis have already studied extensively both in laboratory and clinically. Mego et.al states SDF-1 related with circulating tumour cells and act as chemotactic signal.[18] It also found that SDF-1 is directly correlated with prognosis and tumour progression.[19-23] However, some research found the opposite effect of SDF-1 in which it decreases cancer cell motility, hence, preventing metastasis.[24]

The result of this study, however, confirming that SDF-1 was, in fact, associated with metastasis. It also found that tumour cell itself produce SDF-1 even in greater amount compared with stromal tissue. This result confirms that cancer cell is capable of producing SDF-1 and not just as its target. However, because we found that SDF-1 production by cancer cell exceeded the stromal tissue, further investigation is needed to confirm this finding.

SDF-1/CXCR4 axis play important role in cancer pathogenesis. It controls wide array of cancer characteristic, ranging from maintaining cell proliferation to angiogenesis and cell survival.[19,20,21,25] SDF-1 also required to maintain steady cancer stem cell population. This is not surprising since SDF-1 normally act as regulator of stem cell population in bone marrow.[25]

In relation with metastasis, SDF-1 act as chemotactic factor that attract circulating tumour cell to homing into target organ, often with already formed metastatic niche.[18] SDF-1 also induces cell mobilisation by increasing the expression of β1-integrin.[25] It also induce the expression of several matrix metalloproteinase (MMP) family gene which result in stromal remodelling that favour metastatis.[26] Though not directly related, SDF-1 also plays integral role in angiogenesis. Increase vascular supply not only ensure the oxidative status of cancer mass but also provide easy way for cancer cell to enter vascular system since newly formed blood vessel tend to have fragile and permeable structure.[27]

The aforementioned mechanisms are underlying reason why SDF-1 is often associated with poor prognosis in wide array of cancer.[19-23] In adult acute myelogenous leukemic, SDF-1 was related with lower chemotherapeutic response.[20] Meanwhile in prostate cancer and malignant glioma, it is associated with tumour aggressiveness and progression.[21,22] Overall, it showed that SDF-1 is strong candidate as prognostic biomarker of prognostic in almost every type of cancer.

CONCLUSION

SDF1 over expression in tumour tissue shown to be associated with increased risk distant metastasis in carcinoma cells and it appeared that tumour cell itself is the major source of SDF-1. However, further prospective studies are needed to determine the relative risk SDF-1 over expression in tumour tissue on the occurrence of distant metastases by controlling other factors such as receptors and subtypes of breast cancer.

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