Syndecan-4 levels in bronchoalveolar lavage fluid and serum in non-small cell lung cancer

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ABSTRACT

BACKGROUND Lung cancer has the highest cancer-related mortality rate worldwide. Research has been conducted to improve early detection and markers of predictive value but only focused on the expression of syndecan-4 (SDC4) in serum. Studies in bronchoalveolar lavage (BAL) fluids of non-small cell lung cancer (NSCLC) patients are still limited. This study aimed to evaluate the clinical value of SDC4 in serum and BAL in NSCLC patients.

METHODS Blood serum and BAL fluids were obtained from 44 patients with NSCLC and 41 non-cancer patients as the control. The level of SDC4 was measured. The relationships between SDC4 and clinicopathologic factors were also analyzed.

RESULTS Serum SDC4 levels in NSCLC patients were significantly lower than the control group ($p = 0.002$). Furthermore, the disease stages and serum SDC4 levels had a negative correlation, which was lower in the advanced stage (IIIb/IV) than in the initial stage (I/II/IIIa) ($p = 0.517$). The same results were obtained from BAL fluids SDC4 levels, which were significantly lower in the advanced stage (IIIb/IV) than in the early stage (I/II/IIIa) ($p = 0.007$).

CONCLUSIONS Serum SDC4 levels in NSCLC patients were lower than those of non-cancer patients. They also performed different results in disease stages. SDC4 could be a helpful biomarker in NSCLC.

KEYWORDS lung cancer, non-small cell lung cancer, syndecan-4

Lung cancer is one of the most fatal cancer types, accounting for 1.8 million deaths in 2020, and 84% of all lung cancers are non-small cell lung cancer (NSCLC) types.[1] The high mortality rate of lung cancer is partially due to late diagnoses and limited diagnostic options.[2] Research regarding early detection methods and predictive markers is evolving. However, the mechanisms driving lung tumorigenesis remain poorly understood.[3] The appropriate diagnostic modalities and predictive markers must be chosen based on several factors, including invasiveness, accuracy, risk of complications, and patient comfort.

Traditionally, patient blood samples have been used for non-invasive biomarker analyses. Research regarding the analysis of bronchoalveolar lavage (BAL) fluid for disease evaluation and patient prognosis is taking place based on the vicinity of BAL fluid to tumor cells, the minimally invasive technique, and the less complex protein composition of BAL fluid.[4] The blood and bronchial secretions of patients with lung cancer contain a variety of possible biomarkers; however, only some biomarkers are clinically useful due to their low reproducibility, sensitivity, and specificity.[5–7] Therefore, it is essential to identify and confirm potential biomarkers for the early detection, classification, and staging of lung cancer.

Syndecans (SDCs) are a family of transmembrane heparan sulfate proteoglycans. The four SDC

proteins (SDC1–4) function as co-receptors for cytokines, growth factors, and extracellular matrix (ECM) substances. They are involved in cell adhesion, migration, and growth factor activity,[8] with SDC4 as its central. Although several studies have demonstrated the role of SDC4 in cancer, little is known regarding its expression and role in NSCLC.[9] This study aimed to evaluate the SDC4 levels in serum and BAL fluid and their clinical value in patients with NSCLC.

METHODS

Patients

This cross-sectional study was conducted at the Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi, Indonesia, from April to September 2022. This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Hasanuddin (approval number: 217/UN4.6.4.5.31/PP36/2022).

All patients with suspected cancer were recruited and underwent anatomical and pathological diagnostic procedures (bronchoscopy or transthoracic needle aspiration). If the test results were negative for cancer, the patient was assigned to the control (non-cancer) group. Patients with unclear or ambiguous results were excluded from the study.

Patients aged ≥18 years who were willing to participate in the study were included. However, this study excluded patients who received therapy for small-cell lung cancer prior to enrollment in this study and had cancer in other organs or metastasis to the lung. The patient's age, sex, body mass index (BMI), occupation, smoking status, comorbidities, cancer history, and main symptoms were extracted from the medical records.

The patients were divided into three groups based on their BMI: underweight (<18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), and overweight (25–29.9 kg/m^2).[10] The Brinkman index (IB) was determined as the number of cigarettes smoked per day multiplied by the number of years smoked. Patients were divided into three groups based on the IB: light smokers (IB ≤200), moderate smokers (IB = 201–600), and heavy smokers (IB >600).[11]

Sample collection

This study used samples from the entire accessible population that met the inclusion and exclusion criteria using consecutive sampling techniques. The minimum sample size was 50 and calculation were conducted using the formula for single proportion,[12] with type 1 error of 5% and margin of error of 10%.

A diagnostic bronchoscopy procedure was done to collect BAL fluid samples. The bronchoscope was placed into the bronchus affected by the lesion, and specimens were collected following the infusion of 10–20 ml sterile normal saline.

Blood samples were immediately centrifuged after collection and stored at –80°C prior to analysis. Quantitative enzyme-linked immunosorbent assay kits (LS-F10515, LifeSpanBioSciences, Inc., USA) were used to evaluate the SDC4 levels in the BAL fluid and serum. Standard and test wells containing serum/BAL fluid samples were examined using a microplate assay. The absorbance was measured at 450 nm.

Statistical analysis

Continuous variables with a normal distribution are presented as the mean and standard error of the mean, and those without a normal distribution are presented as the median. Categorical variables are presented as numbers and percentages. The Mann–Whitney *U* test was used to compare continuous variables. The Kruskal–Wallis test and one-way analysis of variance were used to compare multiple groups. If the distributions were not normal, Spearman's Rho test was used to compare the variables. Variables with normal distributions were compared using Pearson's correlation test. All analyses were conducted using SPSS software version 26 (IBM Corp., USA). Statistical significance was set at $p<0.05$.

RESULTS

Patient characteristics

Following the eligibility screening of 144 potential participants, 85 selected patients were divided into NSCLC (44 patients) and control (41 non-cancer patients) groups (Table 1). The relationships between SDC4 and clinicopathologic factors were shown in Table 2.

The serum SDC4 levels were significantly lower in patients with cancer than those without cancer (p = 0.002; Table 2). The BAL fluid were positively correlated with the disease stage (p = 0.007). The SDC4 levels in patients with NSCLC were negatively correlated with the disease stage (p = 0.517) (Table 2).

Table 1. Characteristics of the patients

Characteristics	NSCLC, n (%) (N = 44)	Non-cancer (control), n (%) (N = 41)	Total, n (%) (N = 85)	*p*
Sex				0.251
Male	31 (70)	23 (56)	54 (64)	
Female	13 (30)	18 (44)	31 (36)	
Age (years)				**0.034**
18–50	8 (18)	17 (41)	25 (29)	
≥50	36 (82)	24 (59)	60 (71)	
Main symptom				0.064
Cough	8 (18)	10 (24)	18 (21)	
Hemoptysis	2 (5)	9 (22)	11 (13)	
Dyspnea	20 (45)	12 (29)	32 (38)	
Chest pain	14 (32)	10 (24)	24 (28)	
BMI				0.509
Underweight	6 (14)	11 (27)	17 (20)	
Normal	29 (66)	22 (54)	51 (60)	
Overweight	7 (16)	5 (12)	12 (14)	
Obese	2 (5)	3 (7)	5 (6)	
Smoking status				0.608
Active	24 (55)	18 (44)	42 (49)	
Passive	11 (25)	12 (29)	23 (27)	
None	9 (20)	11 (27)	20 (24)	
Brinkman index				1.000
Mild	4 (17)	6 (33)	10 (24)	
Moderate	8 (33)	6 (33)	14 (33)	
Severe	12 (50)	6 (33)	18 (43)	
Comorbidity				
Tuberculosis	20 (45)	14 (34)	34 (40)	0.400
Diabetes	4 (9)	4 (10)	8 (9)	1.000
Hypertension	10 (23)	7 (17)	17 (20)	0.704
None	10 (23)	16 (39)	26 (31)	-
Thorax CT scan				
Lung mass	39 (89)	22 (54)	61 (72)	**0.001**
Lymphadenopathy	32 (73)	22 (54)	54 (64)	0.110
Metastasis	35 (80)	20 (49)	55 (65)	**0.006**
Mediastinum mass	3 (7)	8 (20)	11 (13)	0.156
Others*	31 (70)	31 (76)	62 (73)	0.772
Bronchoscopy				
Infiltrate lesion/nodule	30 (68)	5 (12)	35 (41)	**0.001**
Lymph gland metastasis	28 (64)	7 (17)	35 (41)	**0.001**
Intrabronchial contralateral metastasis	4 (9)	0 (0)	4 (5)	0.143
Stenosis compression	15 (34)	13 (32)	28 (33)	0.998

BMI=body mass index; CT=computed tomography; NSCLC=non-small cell lung cancer
*Others were atelectasis, cardiomegaly, pulmonary TB, aspergilloma, emphysema, pneumonia, pneumothorax, hydropneumothorax, pleural effusion, empyema, bronchiectasis, hepatomegaly, splenomegaly, and pulmonary fibrosis

Table 2. Level of SDC4 in NSCLC with disease stages and control patients

Sample	BAL fluid (pg/ml) (N = 85)		Serum (pg/ml) (N = 85)	
	Median (min–max)	*p*	Median (min–max)	*p*
NSCLC (N = 44)				
Stages I/II/IIIa	203.4 (147.3–325.5)	**0.007**	113.5 (18.7–220.9)	0.517*
Stages IIIb/IV	100.4 (30.44–413.8)		68.7 (8.7–564.3)	
All NSCLC	113.0 (30.4–413.8)	0.154	68.7 (8.7–564.3)	**0.002***
Non-cancer (control, N = 41)	116.6 (48.4–672.7)	-	145.2 (7.7–504.7)	-

BAL=bronchoalveolar lavage; NSCLC=non-small cell lung cancer; SDC4=syndecan-4
*Mann–Whitney *U* test

DISCUSSION

In this study, serum SDC4 levels were significantly different between patients with NSCLC and those without cancer, though the BAL fluid SDC4 levels did not differ significantly between groups. Low SDC4 levels indicate no inhibition of cell migration and tumor activity; the expression of SDC4 is expected to be reduced as cells undergo malignant transformation. Dysregulation of SDC4 expression contributes to cancer cell development by permitting invasive growth and affecting the metastatic properties of the cells.[13] Low serum SDC4 levels in patients with NSCLC may be associated with the reduced protective ability of this proteoglycan against lung tissue. The results of this study align with previous research findings regarding SDC4 expression in cultured colon carcinoma cells.[13]

Depending on the organ and tumor type, SDCs may affect tumor growth and progression differently. In the current study, patients with NSCLC had lower serum and BAL fluid SDC4 levels than patients without cancer. In addition, the serum and BAL fluid SDC4 levels were negatively correlated with the disease stage. These results are similar to the results of several previous studies regarding the expression of SDC4 in testicular germ cell tumors (TGCT). TGCT was classified into seminomas (less aggressive) and nonseminomatous germ cell tumors (NSGCT, generally more aggressive).[13] The previous study reported that tumor-cell-associated staining for SDC4 was lower in NSGCT than in seminomas. Loss of SDC4 is associated with vascular/lymphatic invasion (p = 0.01), nodal metastasis (p = 0.01), and disease stage (p = 0.01).

In another previous study, fibroblast growth factor 2 therapy resulted in decreased SDC4 expression in melanoma cells, leading to reduced cellular attachment on fibronectin and increased melanoma cell motility, promoting migration.[14] Stronger attachment and decreased cell migration are associated with denser and bigger focal adhesions formed by cells overexpressing SDC4.[8] In addition, SDC4 is upregulated in patients with inflammatory lung injuries. Several *in vitro* and *in vivo* mouse studies have investigated SDC4 levels in the setting of lung cancer. *In vivo* studies have reported a correlation between SDC4 levels and tumor growth and size.[15,16]

Similar to other SDC family members, SDC4 is involved in signal transduction. However, SDC4 is expressed in several cell types.[17] As a proteoglycan, SDC4 interacts with various ligands, including adhesion receptors, growth factors, proteinases, and ECM proteins to initiate downstream signaling pathways. Therefore, SDC4 plays a role in cell adhesion, proliferation, angiogenesis, tumor-associated inflammation, and cell migration.[13,18,19] SDC4 also plays a role in the development and progress of various tumor types, as it is dysregulated in several types. Growth-stimulating and growth-inhibitory effects of SDC4 have been reported. The overexpression of SDC4 has been reported in glioma,[9] melanoma,[14] osteosarcoma,[20] liver cancer,[21] and ovarian carcinoma[9]. However, in colon carcinoma[10] and neuroblastoma,[22] SDC4 expression is reduced. The expression of SDC4 is upregulated in normal breast tissue compared to that in malignant breast tissue.[23] However, SDC4 also plays a role as an anti-migratory and anti-invasive tumor suppressor, based on a previous study that reported significantly lower SDC4 expression in highly metastatic colon carcinoma cells *in vitro*.[13]

Cell migration occurs throughout the cancer formation process, but the initial stage of metastasis is most important during invasion.[23] Metastasis development is a key cause of cancer therapy failure and mortality.[13] The early detection of molecules involved in cancer cell migration can lead to new diagnostic and therapeutic approaches for effective cancer treatment.

Studies regarding the SDC4 role in lung cancer reported that SDC4 is regulated by a disintegrin and metalloproteinase with thrombospondin motif 1, and knockdown of either protein results in the inhibition

of angiogenesis and cell migration via collaboration with matrix metalloproteinase-9 and fibulin-1.[24] The interaction between SDC4 and C-X-C motif chemokine ligand 10 in primary lung fibroblasts also prevents cell migration.[25] In contrast, another study reported that SDC4 promotes invasion and cell migration of A549 lung adenocarcinoma cells in chemotaxis assays and wound healing.[8] SDC4 positively regulates transforming growth factor-mediated epithelial-mesenchymal transition via Snail.

This research still had several limitations, including a cross-sectional research design that was only carried out for a limited period of time. This study compared the SDC4 levels of NSCLC subjects and controls. The control group is non-cancer patients, but have certain disease conditions, so further studies are needed by comparing the SDC4 levels of lung cancer patients with healthy non-cancer subjects. This research is based on *in vitro* studies and *in vivo* mouse models that have much better specimen control. Furthermore, this study did not test SDC and several types of growth factors that could influence the interaction of SDC4 levels on research subjects.

In conclusion, the SDC4 levels in the serum and BAL fluid in NSCLC patients were lower than those of non-cancer patients and are negatively correlated to the disease stage. These results suggest that SDC4 plays a crucial role in the development and progression of NSCLC and can be used as a biomarker for lung cancer. However, further studies are necessary to elucidate the molecular basis of the functions of SDC4 in the setting of lung cancer.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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