Original paper

The prognostic significance of CD63 expression in patients with non-small cell lung cancer

Hyun Min Koh[1], Hyo Jung An[1], Jae Jun Jung[2], Dae Hyun Song[1,3,4]

[1]Department of Pathology, Gyeongsang National University Changwon Hospital, South Korea
[2]Department of Thoracic and Cardiovascular Surgery, Gyeongsang National University Changwon Hospital, South Korea
[3]Gyeongsang National University School of Medicine, South Korea
[4]Gyeongsang Institute of Health Science, South Korea

CD63 has been suggested to participate in tumorigenesis, invasion, and metastasis. In this study, we aimed to investigate the prognostic significance of CD63 expression in non-small cell lung cancer (NSCLC).
CD63 expression was evaluated in 133 cases of NSCLC via immunohistochemical staining using tissue microarray blocks. We assessed the relationship between CD63 expression and clinicopathological characteristics, as well as the prognostic significance of CD63 expression in NSCLC.
CD63 expression was significantly correlated with patient gender ($p < 0.001$), smoking history ($p = 0.020$), histologic type ($p < 0.001$), tumour stage ($p = 0.016$), lymph node metastasis ($p = 0.034$), and TNM stage ($p = 0.007$). A multivariate Cox proportional hazards regression analysis determined low CD63 expression to be an independent factor for unfavourable disease-free survival (DFS) (HR = 2.043, 95% CI: 1.035-4.035, $p = 0.040$), and Kaplan-Meier analysis indicated that the low CD63 expression group showed significantly lower DFS than the high CD63 expression group of patients with NSCLC ($p = 0.019$).
Low CD63 expression might be an unfavourable prognostic factor in patients with NSCLC.

Key words: carcinoma, CD63, lung, non-small cell, prognosis.

Introduction

Non-small cell lung cancer (NSCLC) comprises 85% of lung cancer cases [1]. Most NSCLC patients present with advanced-stage disease and poor prognosis [2]. Despite intensive research efforts in genomics and drug development, the prognosis for patients with NSCLC remains unfavourable, and a substantial number of patients suffer from relapse [2, 3, 4]. Therefore, recognition of differential prognostic groups has been a recent topic of interest in clinical and molecular research, and the discovery of useful biomarkers for accurate prediction of clinical outcomes is essential to improve patient survival [5, 6].

CD63 is a member of the transmembrane-4 superfamily and is involved in many biological processes, including cell growth, adhesion, migration, and differentiation [7, 8]. CD63 is also a tumour suppressor and is associated with malignant progression of melanoma, wherein its expression reduces tumour invasion and metastasis by suppression of tumourigenicity, cellular motility, and matrix-degrading ability in melanoma cells [8, 9, 10]. Several researchers have demonstrated an association between CD63

expression and tumour progression in colon and mammary carcinoma cells and in ovarian cancer tissue [11, 12, 13]. Moreover, recent studies have shown that CD63 knockdown increases tumour invasiveness through upregulation of matrix metalloproteinase expression in oesophageal cancer and tongue squamous cell carcinoma [7, 8].

However, the role of CD63 expression in NSCLC has not been reported extensively. In this study, we assessed the prognostic significance of CD63 expression in NSCLC.

Material and methods

Clinicopathological patient characteristics

We obtained 133 tumour samples from surgically resected NSCLC at the Gyeongsang National University Hospital (Jinju, Korea) between January 2002 and December 2009. NSCLC samples were staged according to the guidelines of the American Joint Committee on Cancer Tumour Node Metastasis (TNM) Classification of Malignant Tumours, eighth edition. The histologic type and differentiation grade of the NSCLC was determined according to the classification system of the World Health Organisation, fourth edition. Clinical and survival data were collected from electronic medical records and National Statistical Office (Seoul, South Korea) records. Disease-free survival (DFS) was defined as the period from the date of surgery to the date of cancer recurrence, and disease-specific survival (DSS) was defined as the period from the date of surgery to the date of death, which was mostly due to NSCLC [14]. Smoking history was defined as smoker or non-smoker (< 100 lifetime cigarettes). This study was approved by the Institutional Review Board of Gyeongsang National University Hospital with a waiver for informed consent (2018-07-029-001).

Tissue microarray construction and immunohistochemistry

Haematoxylin and eosin-stained slides were reviewed, and a core (3 mm in diameter) of the most representative tumour area was prepared from each formalin-fixed paraffin-embedded block. Immunohistochemical staining was performed on 4-μm-thick sections obtained from tissue microarray blocks. Tissue sections were stained with a monoclonal anti-CD63 antibody at a dilution of 1 : 500 (MX-49.129.5, Santa Cruz Biotechnology, CA, USA) using an automated immunostainer (Benchmark Ultra, Ventana Medical Systems Inc., Tucson, AZ, USA). Tumour-infiltrating immune cells were used as the positive control, and the primary antibody was omitted for the negative control.

CD63 expression

The staining intensity of tumour cells was scored as 0 (no staining), 1 (weak staining, light yellow), and 2 (moderate to strong staining, yellowish brown to brown). The proportion of positively stained tumour cells was scored as 1 (0-25%), 2 (26-50%), 3 (51-75%), and 4 (76-100%). A total score was calculated by multiplying the two scores. Finally, each case was classified to a low (< 4) or high (≥ 4) expression group based on the median value. Two pathologists individually evaluated all slides.

Statistical analysis

The relationship of CD63 expression with clinicopathological characteristics was assessed using Pearson's χ^2. The prognostic value of clinicopathological characteristics for DFS and DSS was evaluated by the Cox proportional hazard regression method. DFS and DSS were analysed by the Kaplan-Meier method with the log-rank test. A p-value less than 0.05 was considered statistically significant. Analyses were performed using IBM SPSS ver. 25.0 (IBM Corp., Armonk, NY, USA).

Results

Clinicopathological patient characteristics

The median age of diagnosis was 66 years (range: 31-77 years). Among all the enrolled patients, 111 (83.5%) were male, and 86 (65.2%) had smoking experience. The histologic types of the tumours were as follows: 96 (72.2%) squamous cell carcinoma (SCC) and 37 (27.8%) adenocarcinoma (ADC). The most common histological feature of SCC was moderately differentiated in 59 (61.4%), and ADC was acinar type in 15 (40.5%). The TNM stages were as follows: 57 (42.8%) were stage I, 54 (40.6%) were stage II, 19 (14.3%) were stage III, and three (2.3%) were stage IV (Table I).

The relationship between CD63 expression and clinicopathological characteristics

CD63 expression revealed a granular pattern in the cytoplasm of tumour cells. Among all cases, 99 (74.4%) showed low expression and 34 (25.6%) showed high expression. The representative images of CD63 expression are shown in Fig. 1A-D. CD63 expression was significantly correlated with patient gender ($p < 0.001$), smoking history ($p = 0.020$), histological type ($p < 0.001$), tumour stage ($p = 0.016$), lymph node metastasis ($p = 0.034$), and TNM stage ($p = 0.007$), but not with patient age and distant metastasis (Table I). Low CD63 expression was more frequent in men than in women, in smokers than in

Table I. Relationship between CD63 expression and clinicopathological characteristics

Characteristic	Number of cases	CD63 expression		p-value
	(n = 133)	Low (n = 99, 74.4%)	High (n = 34, 25.6%)	
Age (years)				0.863
< 65	57 (42.9)	42	15	
≥ 65	76 (57.1)	57	19	
Sex				< 0.001
Male	111 (83.5)	91	20	
Female	22 (16.5)	8	14	
Smoking history				0.020
Non-smoker	46 (34.8)	29	17	
Smoker	86 (65.2)	70	16	
Histologic type				< 0.001
SCC	96 (72.2)	87	9	
ADC	37 (27.8)	12	25	
Tumour stage				0.016
T1, T2	101 (75.9)	70	31	
T3, T4	32 (24.1)	29	3	
Lymph node metastasis				0.034
Absent	90 (67.7)	62	28	
Present	43 (32.3)	37	6	
Distant metastasis				0.099
Absent	130 (97.7)	98	32	
Present	3 (2.3)	1	2	
TNM stage				0.007
I	57 (42.8)	36	21	
II	54 (40.6)	44	10	
III	19 (14.3)	18	1	
IV	3 (2.3)	1	2	

Values are presented as numbers (%). ADC – adenocarcinoma, SCC – squamous cell carcinoma, TNM – tumour node metastasis.

non-smokers, in SCC than in ADC, in higher tumour stage than in lower stage, in present lymph node metastasis than in absent lymph node metastasis, and in higher TNM stage than in lower TNM stage.

CD63 expression and survival analysis

Kaplan-Meier analysis demonstrated that the low CD63 expression group showed a significantly lower DFS compared to the high CD63 expression group (p = 0.019; Fig. 2A). Multivariate Cox proportional hazards regression analysis determined that low CD63 expression is an independent factor for unfavourable DFS (hazard ratio [HR]: 2.043, 95% CI: 1.035-4.035, p = 0.040) (Table II). However, the DSS rate was not found to be significantly different between the groups with high and low CD63 expression (p = 0.099; Fig. 2B), and low CD63 expression had no valuable prognostic effect on DSS (Table II).

Discussion

In this study, we demonstrate that low CD63 expression can be a useful prognostic factor for unfavourable DFS in patients with NSCLC. We also found that low CD63 expression in higher tumour stage, present lymph node metastasis, and higher TNM stage was significantly more prevalent than that in lower tumour stage, absent lymph node metastasis, and lower TNM stage, which suggests a possible association between low CD63 expression and patient survival.

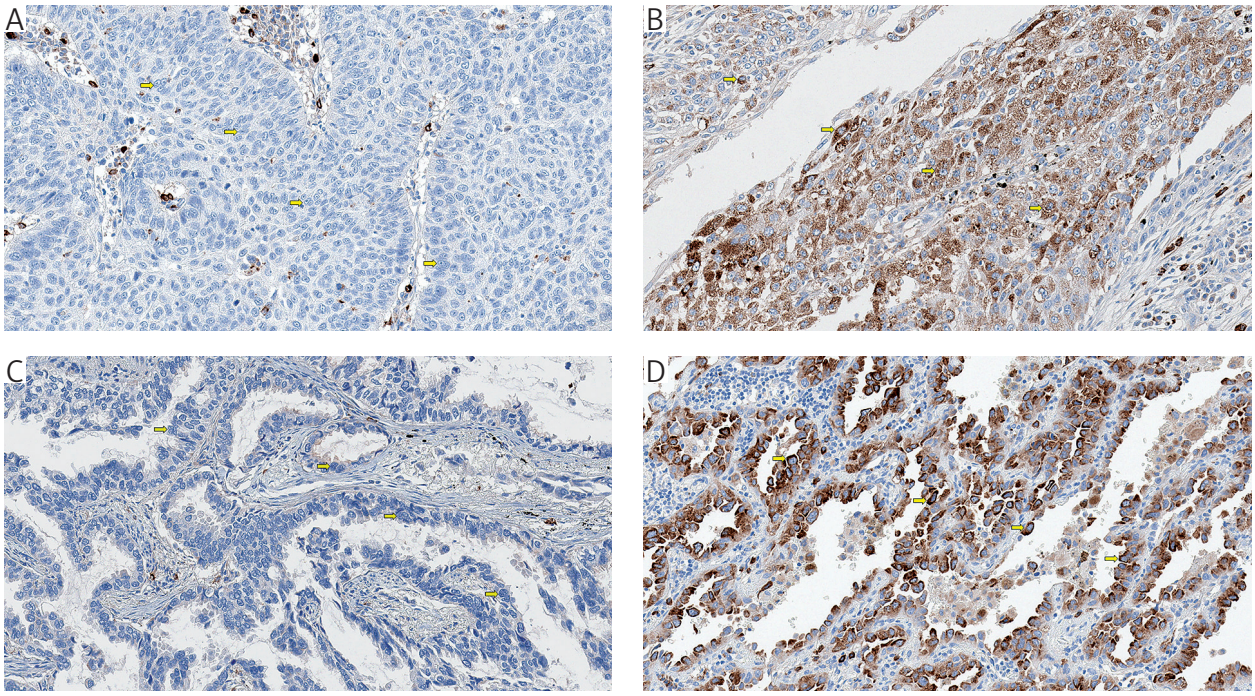

Fig. 1. CD63 expression in non-small cell lung cancer. Low and high expression in squamous cell carcinoma (**A, B**) and in adenocarcinoma (**C, D**), respectively (marked with arrows, immunohistochemical staining for CD63, original magnification: 200×)

Table II. Cox proportional regression analysis of DFS and DSS for NSCLC patients (n = 133)

Variables	Univariate analysis				Multivariate analysis			
	DFS		DSS		DFS		DSS	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Age (years) (< 65 vs. ≥ 65)	1.400 (0.843-2.325)	0.193	1.244 (0.717-2.158)	0.437				
Sex (male vs. female)	0.565 (0.257-1.239)	0.154	0.358 (0.129-0.992)	0.048			0.555 (0.178-1.726)	0.309
Smoking (non-smoker vs. smoker)	0.891 (0.533-1.488)	0.658	1.029 (0.583-1.817)	0.922				
Histologic type (SCC vs. ADC)	0.540 (0.287-1.013)	0.055	0.427 (0.201-0.908)	0.027			0.514 (0.222-1.189)	0.120
TNM stage (I, II vs. III, IV)	2.201 (1.246-3.887)	0.007	1.866 (0.979-3.557)	0.058	2.035 (1.147-3.608)	0.015	1.916 (1.000-3.671)	0.050
CD63 expression (high vs. low)	2.193 (1.114-4.318)	0.023	1.809 (0.881-3.713)	0.106	2.043 (1.035-4.035)	0.040		

ADC – adenocarcinoma; CI – confidence interval; DFS – disease-free survival; DSS – disease-specific survival; HR – hazard ratio; NSCLC – non-small cell lung cancer; SCC – squamous cell carcinoma; TNM – tumour-node-metastasis

Moreover, low CD63 expression in men, smokers, and SCC was significantly more frequent than that in women, non-smokers, and ADC. However, there are limitations to the interpretation of these results because our cases mainly included men, smokers, and SCC.

Kown *et al*. [15] reported that negative CD63 expression was significantly correlated with patient gender, histologic type, tumour stage, and TNM stage in NSCLC, which is consistent with our results. They reported that the negative CD63 expression group showed poorer survival than the positive group in lung ADC [15]. These results are partly concordant with our results. However, there is a slight difference in that our results revealed low CD63 expression to be significantly associated with poor DFS, but not with DSS in NSCLC, using Kaplan-Meier analysis as well as Cox proportional multivariate analysis. We also identified for the first time that CD63 is expressed in SCC, compared to the previous study [15].

We assessed DFS in groups with lower TNM stage (I and II) and higher TNM stage (III and IV), respectively, because an association between decreased CD63 expression and tumour progression or prognostic impact might be induced in the late stages of tumour development or earlier clinical stages, according to previous reports [15, 16, 17]. When we evaluated only the lower TNM stage, low CD63 expression was still relevant for DFS ($p = 0.014$) (Fig. 2C) in Kaplan-Meier analysis, but not in higher TNM stage.

CD63 was originally discovered as a tumour suppressor in melanoma cells [16, 18, 19, 20]. It was thought that reduced CD63 expression enhances cell motility, invasive ability, and metastatic capacity with the involvement of integrins and extracellular matrix molecules, especially matrix metalloproteases, indicating an inverse correlation between CD63 expression and tumour progression [17]. However, the role of CD63 has recently been reassessed because CD63 was found to interact with tissue inhibitor of metalloproteinase on the cell surface, which may act as a pro-tumorigenic and pro-metastatic factor [21]. Some studies have reported that CD63 expression may cause tumour progression and lower survival [22, 23, 24]. Therefore, we recommend extensive studies to better understand the exact role of CD63 in NSCLC.

In summary, this study is the first report to reveal an association between CD63 expression and NSCLC including SCC, which indicates that low CD63 expression might be an unfavourable prognostic factor in patients with NSCLC.

The authors declare no conflict of interest.

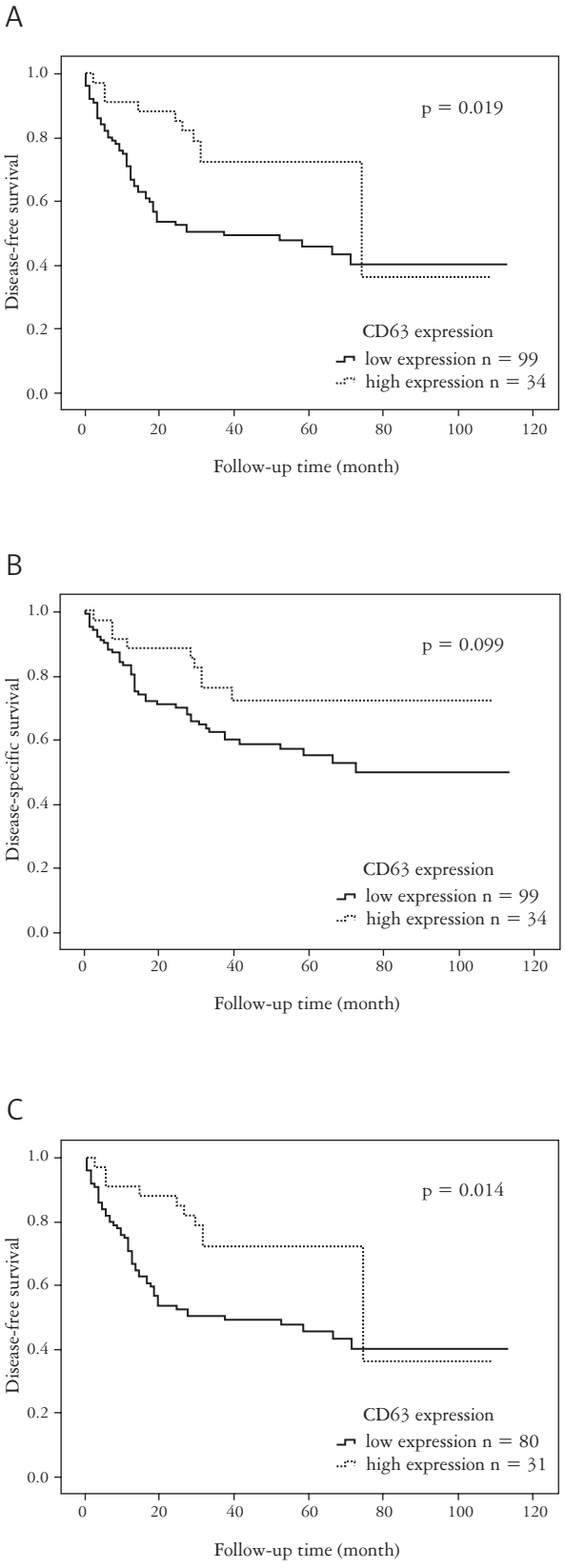

Fig. 2. Survival curves based on CD63 expression in patients with non-small cell lung cancer. **A**) Disease-free survival and **B**) disease-specific survival in all enrolled patients, and **C**) disease-free survival in patients with lower TNM stage (I and II)

References

1. Li L, Sun Y, Feng M, et al. Clinical significance of bloodbased miRNAs as biomarkers of nonsmall cell lung cancer. Oncol Lett 2018; 15: 8915-8925.
2. Ku BM, Heo MH, Kim JH, et al. Molecular screening of small biopsy samples using next-generation sequencing in korean patients with advanced non-small cell lung cancer: Korean lung cancer consortium (KLCC-13-01). J Pathol Transl Med 2018; 52: 148-156.
3. Jo YM, Park TI, Lee HY, et al. Prognostic significance of aquaporin 5 expression in non-small cell lung cancer. J Pathol Transl Med 2016; 50: 122-128.
4. Campa MJ, Wang MZ, Howard B, et al. Protein expression profiling identifies macrophage migration inhibitory factor and cyclophilin a as potential molecular targets in non-small cell lung cancer. Cancer Res 2003; 63: 1652-1656.
5. Yun S, Sun PL, Jin Y, et al. Aquaporin 1 is an independent marker of poor prognosis in lung adenocarcinoma. J Pathol Transl Med 2016; 50: 251-257.
6. Bandres E, Bitarte N, Arias F, et al. microRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells. Clin Cancer Res 2009; 15: 2281-2290.
7. Lai X, Gu Q, Zhou X, et al. Decreased expression of CD63 tetraspanin protein predicts elevated malignant potential in human esophageal cancer. Oncol Lett 2017; 13: 4245-4251.
8. Liu W, Li X, Zhu X, et al. CD63 inhibits the cell migration and invasion ability of tongue squamous cell carcinoma. Oncol Lett 2018; 15: 9033-9042.
9. Jang H, Lee H. A decrease in the expression of CD63 tetraspanin protein elevates invasive potential of human melanoma cells. Exp Mol Med 2003; 35: 317-323.
10. Kang M, Ryu J, Lee D, et al. Correlations between transmembrane 4 L6 family member 5 (TM4SF5), CD151, and CD63 in liver fibrotic phenotypes and hepatic migration and invasive capacities. PLoS One 2014; 9: e102817.
11. Sordat I, Decraene C, Silvestre T, et al. Complementary DNA arrays identify CD63 tetraspanin and α3 integrin chain as differentially expressed in low and high metastatic human colon carcinoma cells. Lab Invest 2002; 82: 1715-1724.
12. Sauer G, Kurzeder C, Grundmann R, et al. Expression of tetraspanin adaptor proteins below defined threshold values is associated with in vitro invasiveness of mammary carcinoma cells. Oncol Rep 2003; 10: 405-410.
13. Zhijun X, Shulan Z, Zhuo Z. Expression and significance of the protein and mRNA of metastasis suppressor gene ME491/CD63 and integrin alpha5 in ovarian cancer tissues. Eur J Gynaecol Oncol 2007; 28: 179-183.
14. Song DH, Ko GH, Lee JH, et al. Prognostic role of myoferlin expression in patients with clear cell renal cell carcinoma. Oncotarget 2017; 8: 89033-89039.
15. Kwon MS, Shin S, Yim S, et al. CD63 as a biomarker for predicting the clinical outcomes in adenocarcinoma of lung. Lung Cancer 2007; 57: 46-53.
16. Kondoh M, Ueda M, Ichihashi M, et al. Decreased expression of human melanoma-associated antigen ME491 along the progression of melanoma pre-canceroses to invasive and metastatic melanomas. Melanoma Res 1993; 3: 241-245.
17. Pols MS, Klumperman J. Trafficking and function of the tetraspanin CD63. Exp Cell Res 2009; 315: 1584-1592.
18. Radford KJ, Mallesch J, Mersey P. Suppression of human melanoma cell growth and metastasis by the melanoma-associated antigen CD63 (ME491). Int J Cancer 1995; 62: 631-635.
19. Radford KJ, Thorne RF, Hersey P. CD63 associates with transmembrane 4 superfamily members, CD9 and CD81, and with β1 integrins in human melanoma. Biochem Biophys Res Commun 1996; 222: 13-18.
20. Radford KJ, Thorne RF, Hersey P. Regulation of tumor cell motility and migration by CD63 in a human melanoma cell line. J Immunol 1997; 158: 3353-3358.
21. Seubert B, Cui H, Simonavicius N, et al. Tetraspanin CD 63 acts as a pro-metastatic factor via β-catenin stabilization. Int J Cancer 2015; 136: 2304-2315.
22. Cui H, Seubert B, Stahl E, et al. Tissue inhibitor of metalloproteinases-1 induces a pro-tumourigenic increase of miR-210 in lung adenocarcinoma cells and their exosomes. Oncogene 2015; 34: 3640-3650.
23. Rorive S, Lopez XM, Maris C, et al. TIMP-4 and CD63: New prognostic biomarkers in human astrocytomas. Mod Pathol 2010; 23: 1418-1428.
24. Toricelli M, Melo FH, Hunger A, et al. Timp1 promotes cell survival by activating the PDK1 signaling pathway in melanoma. Cancers 2017; 9: E37.

Address for correspondence

Dae Hyun Song
Department of Pathology
Gyeongsang National University Changwon Hospital
South Korea
e-mail: golgy@hanmail.net