ORIGINAL PAPER

THE PROGNOSTIC SIGNIFICANCE OF CD63 EXPRESSION IN PATIENTS WITH NON-SMALL CELL LUNG CANCER

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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 tumorigenicity, 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 χ². 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...
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)

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


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