this study on the basis of the hypothesis that Psf3 may also be a marker for cancer stem cells. Previous study has shown that all GINS components overexpression in intrahepatic cholangiocarcinoma tissues and Psf3 expression in human colon carcinoma [15,17]. To our knowledge, this is the first study to detect Psf3 expression in lung adenocarcinoma and to show that Psf3 expression might be a useful prognostic marker for assessing patient survival in lung adenocarcinoma.

In this study, we performed IHC of surgically resected lung adenocarcinoma specimens to determine the Psf3 status in cancer cells and cancer tissues clinically. The results of IHC revealed that the protein was specifically localized to the nuclei of cancer cells. The staining pattern for Psf3 was quite characteristic and reproducible.

IHC revealed that Psf3 expression was increased in 21.6% (27/125) of the lung adenocarcinoma specimens but not in normal lung tissues (Fig. 1A). In order to elucidate the role of increased Psf3 expression on the prognosis of patients with lung adenocarcinoma, a prognostic analysis was carried using the patients' follow-up data. Survival analysis revealed that the OS rate in patients positive for increased Psf3 expression was notably lower than that of the Psf3-low positive group (Fig. 2A). These findings indicated that increased Psf3 expression low positively affected the clinical course and was correlated with malignant behavior of tumors. The significance of Psf3 expression in cancer cells on these clinical features was also supported by our analysis of the relationship between Psf3 expression and clinicopathological characteristics of 125 patients (Table 2). Cox multivariate analysis indicated that increased Psf3 expression was the most significant predictor of poor prognosis, rather than the pathological T factor or N factor. Furthermore, a prognostic analysis that included only stage I cases revealed that the OS rate of the Psf3-positive group was significantly lower than that of the Psf3-low positive group. These findings suggest that increased Psf3 expression may be used as a reference index for molecular staging of patients with a high risk of death and thereby likely to benefit from intensive adjuvant therapy.

What is the basis of the relationship between increased Psf3 expression and poor prognosis? We believe that increased Psf3 expression may be related to cancer cell proliferation because Psf3 was required in the early stage of DNA replication, along with other GINS members [11–14]. In this study, Ki67 (MIB-1) expression index was used as an indicator of cell proliferation. The Mann–Whitney *U*-test indicated that the Ki67 index level was higher in the Psf3-positive group than in the Psf3-low positive group (Fig. 3).

Table 3
Univariate and multivariate analysis of the association between the overall survival of 125 patients with lung adenocarcinoma and other candidates for biomarkers by Cox proportional hazard models.

Variable	Hazard ratio	95% confidence interval	*P*-value
Univariate			
Ki67	1.02	1.00–1.05	0.0412*
γ-H2AX	1.73	0.75–4.00	0.193
p53	1.17	0.51–2.69	0.699
Necl-5	1.82	0.49–6.74	0.368
Wip1	7.32	1.56–34.2	0.0113*
Psf3	6.91	2.70–17.6	0.0001*
Multivariate			
Ki67	0.97	0.93–1.01	0.266
γ-H2AX	0.77	0.22–2.73	0.695
p53	0.80	0.23–2.73	0.726
Necl-5	1.42	0.25–7.95	0.688
Wip1	4.77	0.89–25.5	0.067
Psf3	12.1	2.51–58.3	0.0019*

γ-H2AX, phosphorylated H2AX; p53, tumor protein p53; Necl-5, Nectin-like molecule-5; Wip1, Wild-type p53-induced phosphatase 1.
* Significant *P*-value.

Subsequently, we examined the status of cell proliferation at the Psf3-positive sites in cancer tissues in order to investigate the role of Psf3 expression on cancer cell biology, by using serial sections of cancer tissues. The ratio of Ki67-positive cancer cells was extremely higher in areas with excessive nuclear staining of Psf3 (Fig. 1C and D). This finding indicated that the proliferation potential was specifically higher at these areas. The accumulation of Psf3 in the nuclei of the cancer cells might be indicative of its role in the acceleration of cancer cell proliferation.

Additionally, we compared the staining patterns of Ki67 and Psf3 in the same serial sections and found that the staining patterns of the 2 proteins were different. While almost all nuclei of cancer cells were stained with the Psf3 antibody in the clustered area (Fig. 1C), Ki67 staining was observed in a "scattered pattern" and the ratio of Ki67-positive nuclei was ≤50% (Fig. 1D). During the cell cycle of eukaryotic cells, Ki67 is expressed at increased levels during the S phase, with the maximum expression in the M phase [21,22]. In other words, the cells with nuclear staining for Ki67 antibody are in the middle of cell division. Thus, the staining pattern ("scattered pattern") of Ki67 detected in our study is consistent with the biological role of Ki67. Because cancer cells in each phase are randomly distributed in cancer tissues and Ki67 level in nuclei was functionally increasing and decreasing in cell cycle dependent manner, the "scattered" pattern of Ki67 staining was observed.

On the other hand, staining for Psf3 showed a pattern different from that of staining for Ki67. In some tumor tissues, the nuclei of cancer cell were stained in a "scattered pattern" (judged as low positive for Psf3 in this study) similar to that observed for Ki67. In other cases, clustered nuclear staining (judged as positive for Psf3 in this study) was observed in some areas of tumor tissues. These findings were suggestive of the aberrant accumulation of Psf3 in the nuclei of the clustered area. While Psf3 staining in a "scattered pattern" seemed to be dependent on the cell cycle, similar to the case with Ki67, the clustered pattern of staining for Psf3 was persistently observed in nuclei of cancer cells out of the cell cycle.

We previously performed IHC analysis of 10 cancer-related proteins by using the same paraffin-embedded specimens examined in this study and compared the expression levels of these proteins and Psf3 to determine the strongest predictor of poor prognosis. Statistical analysis revealed that Psf3 was the most promising candidate as a marker of poor prognosis (Table 3). Immunohistochemical investigation of Psf3 expression in lung cancer specimens seems to be a useful tool because of the following reasons: (1) its statistically significant association with poor prognosis, (2) the objectivity and reproducibility of the test for positive expression because of the specific and sharply defined staining pattern, and (3) the existence of biological and pathological evidence regarding Psf3.

In conclusion, our results suggested that increased Psf3 expression in cancer cells in primary lung adenocarcinoma plays an important role in the progression of lung adenocarcinoma and acts as a factor low positively affecting the prognosis of patients. These results suggested that Psf3 could be used as a reference index for the molecular staging to select patients at high risk of death and relapsed patients who may benefit from intensive adjuvant therapy.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Article

XRCC3 Gene Polymorphism Is Associated with Survival in Japanese Lung Cancer Patients

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Abstract: We focused on *OGG1* Ser326Cys, *MUTYH* Gln324His, *APEX1* Asp148Glu, *XRCC1* Arg399Gln, and *XRCC3* Thr241Met and examined the relationship between the different genotypes and survival of Japanese lung cancer patients. A total of 99 Japanese lung cancer patients were recruited into our study. Clinical data were collected, and genotypes of the target genes were identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Survival analysis to verify the impact of these gene polymorphisms on the clinical outcome of lung cancer showed that lung squamous cell carcinoma patients with the Thr/Met genotype at *XRCC3* had a significantly shorter survival time than those with the Thr/Thr genotype (13 months *versus* 48 months; log-rank test, $p < 0.0001$). Cox regression analysis showed that the carriers of *XRCC3* genotypes were at a significantly higher risk [adjusted hazard ratio (HR) = 9.35, 95% confidence interval (CI) = 2.52–34.68, $p = 0.001$; adjusted HR = 9.05, 95% CI = 1.89–44.39, $p = 0.006$]. Our results suggest that *XRCC3* Thr241Met may act as a favorable prognostic indicator for lung squamous cell carcinoma patients.

Keywords: gene polymorphisms; lung cancer; survival; DNA repair; XRCC3

Abbreviations: NER, nucleotide excision repair; BER, base excision repair; DSBR, double-strand break repair; OGG1, 8-oxoguanine DNA glycosylase; MUTYH/MYH, Mut Y homolog; APEX1/APE1, apurinic/apyrimidinic endonuclease-1; XRCC1, X-ray repair cross-complementing group 1; XRCC3, X-ray repair cross-complementing group 3; CI, confidence interval; NSCLC, non-small-cell lung cancer; SD, standard deviation; HR, hazard ratio; MST, median survival time.

1. Introduction

Lung cancer is a major cause of cancer mortality worldwide. The 5-year survival rate for lung cancer, particularly non-small-cell lung cancer (NSCLC), remains at less than 20% [1,2]. Genetic factors are considered to influence the outcome of lung cancer. Among genetic factors, DNA repair capacity is an important factor. The reason appears to be that DNA repair pathways, including nucleotide excision repair (NER), base excision repair (BER), and double-strand break repair (DSBR), play an important role in maintaining genetic stability through different pathways [3,4]. It is also possible that DNA repair capacity can affect the survival of lung cancer patients.

Four key proteins in the BER pathway are 8-oxoguanine DNA glycosylase (OGG1), Mut Y homolog (MUTYH/MYH), apurinic/apyrimidinic endonuclease-1(APEX1/APE1), and X-ray repair cross-complementing group 1 (XRCC1). Among the various DNA repair pathways, BER is considered to play a key role in removing DNA damage resulting from exposure to various endogenous and exogenous carcinogens. OGG1 and MUTYH recognize and remove the misincorporated oxidized nucleotide 8-OHdG and the adenine paired with 8-OHdG, respectively, and also prevent the occurrence of these events. *OGG1* Ser326Cys is associated with the risk of lung cancer [5]. We have reported that *MUTYH* Gln324His was associated with increased risk of lung and colorectal cancers [6,7]. APEX1, the most stable product of oxidative DNA damage, exhibits 3'-phosphodiesterase activity that removes the abasic sites from cleaved DNA through OGG1 and MUTYH proteins [8]. Recently, we reported that genetic polymorphisms of *APEX1* in DNA repair pathways contributed to lung cancer susceptibility, which was dependent on smoking status [6,9]. XRCC1 encodes a protein that complexes with DNA ligase to repair DNA gaps resulting from BER, and a polymorphism at codon 399 Arg to Gln of *XRCC1* is associated with the risk of lung cancer.

In the DSBR pathway, the X-ray repair cross-complementing group 3 (XRCC3) is integral to DNA double-strand break recombination repair, and a polymorphism in codon 241 (Thr and Met) of *XRCC3* has been associated with the level of bulky DNA adducts in leukocytes of healthy subjects [10].

Recently, there has been increasing evidence that reduced DNA repair capacity resulting from genetic polymorphisms of various DNA repair genes is associated with improved survival with platinum-based chemotherapy [11,12].

To our knowledge, few previous studies have examined the effect of these polymorphisms on the association between outcome and lung cancer in Japanese patients without chemotherapy. To determine the significance of these polymorphisms, we focused on *OGG1* Ser326Cys (rs1052133), *MUTYH* Gln324His (rs3219489), *APEX1* Asp148Glu (rs1130409), *XRCC1* Arg399Gln (rs25487), and

XRCC3 Thr241Met (rs861539) and examined the relationship between the different genotypes and the survival of Japanese lung cancer patients.

2. Results

The distribution of characteristics and clinical features of 99 lung cancer patients are shown in Table 1. The average age [± standard deviation (SD)] of the patients was 66.3 ± 9.3 years, the average tumor size (± SD) was 36.4 ± 9.3 mm, and the average pack-years (±SD) was 34.7 ± 31.9 years. Histological analysis of samples from these patients showed that 65.4% had adenocarcinoma, 29.8% had squamous cell carcinoma, and 4.8% had other types of carcinoma. A total of 52 patients had died. The overall median survival time (MST) was 63 months. Region of metastasis mainly involved lung, bone, or mediastinum lymph node. As shown in Table 1, males; patients ≥65 years of age; whose histological subtype was squamous cell carcinoma; those who smoked; and those who had advanced cancer (stage III and IV), and who had T stage (T2-4), lymph node metastasis, or recurrence had significantly shorter MSTs (log-rank test, $p < 0.05$). The median survival was 52 months for males and 81 months for females (log-rank test, $p = 0.001$).

Table 1. Demographic and Clinical Characteristics of Lung Cancer Patients.

Variable	Patients *n*	Median Survival (Months)	Log-rank *p* value	Adjusted HR (95% CI)	*p* value
Gender					
Male	65	52	-	1.00	-
Female	34	81	0.001	0.32 (0.16–0.65)	0.001
Age (years)					
<65	36	78		1.00	
≥65	63	52	0.002	2.75 (1.41–5.36)	0.003
Histological subtype					
adenocarcinoma	65	67	-	1.00	-
squamous cell carcinoma	29	49	0.020	1.93 (1.10–3.40)	0.023
others	5	41	-	-	-
Smoking status					
Non-smokers (Pack-years = 0)	31	80	-	1.00	-
Smokers (Pack-years > 0)	67	53	0.002	3.02 (1.47–6.22)	0.003
No information	1	-	-	-	-
Stage					
I & II	74	69	-	1.00	-
III & IV	20	41	0.001	2.60 (1.42–4.75)	0.002
No information	5	15	-	-	-
T stage					
T1	39	82	-	1.00	-
T2&T3&T4	55	49	<0.0001	3.68 (1.88–7.23)	<0.0001
No information	5	15	-	-	-
Lymph node metastasis					
N0	66	68	-	1.00	-
N1&N2	28	49	0.030	1.87 (1.05–3.33)	0.034
No information	5	15	-	-	-
Distant metastasis					
M0	89	66	-	1.00	-
M1	5	50	0.783	1.18 (0.37–3.79)	0.784
No information	5	14	-	-	-
Recurrence					
No	50	83		1.00	
Yes	44	43	<0.0001	6.15 (3.09–12.24)	<0.0001
No information	5	4	-	-	-

Multiple Cox regression analysis suggested that the risks of death from lung cancer were increased in patients with stages III and IV than in those with stages I and II (HR = 2.60, 95% CI = 1.42–4.75, p = 0.002), especially who had T stage (T2-4) and lymph node metastasis (HR = 3.68, 95% CI = 1.88–7.23, $p < 0.0001$ for T stage (T2&T3&T4); HR = 1.87, 95% CI = 1.05–3.33, $p = 0.034$ for lymph node metastasis; HR = 1.18, 95% CI = 0.37–3.79, $p = 0.784$ for distant metastasis) . This analysis also showed that patients who were older (≥65 years of age), who smoked, whose histological subtype was squamous cell carcinoma, and who had recurrence had increased risks of death (HR = 2.75, 95% CI = 1.41–5.36, $p = 0.003$ for ≥65 years of age; HR = 1.93, 95% CI = 1.10–3.40, $p = 0.023$ for squamous cell carcinoma; HR = 3.02, 95% CI = 1.47–6.22, $p = 0.003$ for smoking status and HR = 6.15, 95% CI = 3.09–12.24, $p < 0.0001$ for recurrence). Females had significantly longer survival than males (HR = 0.32, 95% CI = 0.16–0.65, $p = 0.001$).

The associations between genotypes of 5 SNPs and survival of lung cancer patients are shown in Table 2. No associations were found between polymorphisms of these 5 genes and the overall survival of these patients. In the Cox regression mode, after adjusting for age, gender, tumor stage, metastasis, and recurrence, no associations were found between polymorphisms of these 5 genes and the overall risks of death of these patients.

Table 2. DNA Repair Gene Polymorphisms and Patient Survival.

Genotype	Patients	MST	Log-rank	HR (95% CI)	Adjusted HR		
	n	(mon)	*p* value		*p* value	(95% CI) [a]	*p* value
OGG1							
Ser/Ser	25	58		1.00	-	1.00	-
Ser/Cys	50	70	0.910	0.88 (0.45–1.72)	0.712	0.96 (0.44–2.10)	0.927
Cys/Cys	24	63		0.99 (0.46–2.10)	0.972	0.90 (0.40–2.06)	0.808
Ser/Cys, Cys/Cys	74	66	0.784	0.92 (0.49–1.72)	0.786	0.94 (0.47–1.89)	0.854
MUTYH							
Gln/Gln	20	58		1.00	-	1.00	-
Gln/His	53	63	0.914	1.13 (0.55–2.33)	0.731	0.96 (0.42–2.17)	0.919
His/His	26	70		1.02 (0.45–2.32)	0.972	0.85 (0.33–2.18)	0.851
Gln/His, His/His	79	63	0.797	1.09 (0.55–2.18)	0.798	0.93 (0.42–2.03)	0.845
APEX							
Asp/Asp	40	58		1.00	-	1.00	-
Asp/Glu	48	62	0.649	0.88 (0.50–1.55)	0.652	1.02 (0.55–1.88)	0.963
Glu/Glu	11	71		0.61 (0.21–1.78)	0.37	0.51 (0.15–1.76)	0.289
Asp/Glu, Glu/Glu	59	64	0.505	0.83 (0.48–1.44)	0.508	0.91 (0.50–1.66)	0.766
XRCC1							
Arg/Arg	44	62	-	1.00	-	1.00	-
Arg/Gln	49	56	0.162	1.20 (0.69–2.08)	0.524	0.85 (0.46–1.55)	0.588
Gln/Gln	6	91		0.22 (0.03–1.62)	0.136	0.37 (0.05–2.87)	0.342
Arg/Gln, Gln/Gln	55	60	0.897	1.04 (0.60–1.79)	0.898	0.80 (0.44–1.46)	0.470
XRCC3							
Thr/Thr	88	66	-	1.00	-	1.00	
Thr/Met	11	28	0.202	1.67 (0.75–3.72)	0.209	1.94 (0.83–4.53)	0.128
Met/Met	0	-	-	-	-	-	-
Thr/Met, Met/Met	11	28	0.202	1.67 (0.75–3.72)	0.209	1.94 (0.83–4.53)	0.128

a: HR adjusted for gender, age, smoking history, disease stage, metastasis, and reccurence.

Table 3 summarizes the genotype distribution for lung adenocarcinomas and squamous cell carcinomas. No associations were found between polymorphisms of these 5 genes and the risks of death for adenocarcinoma patients. For squamous cell carcinoma, patients with Thr/Met genotype at *XRCC3* showed a significantly shorter survival time than those with the Thr/Thr genotype (13 months *versus* 48 months; log-rank test, $p < 0.0001$) (Figure 1). Cox regression analysis showed that carriers of *XRCC3* had a significantly a higher risk (crude HR = 9.35, 95% CI = 2.52–34.68, $p = 0.001$; adjusted HR = 9.05, 95% CI = 1.89–44.39, $p = 0.006$).

3. Discussion

In this study, we assessed the *OGG1* Ser326Cys, *MUTYH* Gln324His, *APEX1* Asp148Glu, *XRCC1* Arg399Gln, and *XRCC3* Thr241Met gene polymorphisms that may influence DNA repair capacity and their association with the overall survival of lung cancer patients. The polymorphisms chosen for this study have also been shown to have functional significance and may be responsible for a low DNA repair capacity phenotype that is characteristic of cancer patients [13,14]. To our knowledge, this is the first report on these DNA repair gene polymorphisms in relation to survival without chemotherapy in Japanese lung cancer patients. In a previous study of Japanese patients, Takenaka *et al.* reported that the *ERCC1 C8092A* polymorphism may influence NSCLC prognosis regardless of ERCC1 protein expression and platinum sensitivity [15]. We explored the genotypes as well as pathological features of lung cancer patients in terms of their overall survival. In this study, *XRCC3* Thr241Met might be an independent prognostic factor in squamous cell carcinoma. The adjusted HR for *XRCC3* was 9.05 ($p = 0.006$), with the *XRCC3* group being significant. This *XRCC3* variant genotype was associated with significantly decreased survival in squamous cell carcinoma. In contrast, we observed that the patients carrying none of the adverse genotypes (*OGG1* Ser326Cys, *MUTYH* Gln324His, *APEX1* Asp148Glu, and *XRCC1* Arg399Gln) had much better survival than those carrying variant alleles. For BER genes, several studies reported that variant alleles of *XRCC1* 399 and *XRCC1* variant genotypes are significantly associated with poor survival [11,12,16–19]. In China, *ERCC1* and *XRCC1* were associated with the survival of non-smoker female lung adenocarcinoma patients [20]. Consistent with our study, Penas *et al.* showed that *XRCC 3* is strongly associated with the survival of NSCLC patients treated with cisplatin/gemcitabine [21]. They suggested that the reduced efficacy of the XRCC3 protein, a consequence of the polymorphic variant, may have resulted in an impaired ability to repair cisplatin DNA damage [22]. Another report showed that the *XRCC3* Met/Met genotype was significantly associated with increased risk of death among all patients, particularly males by univariate and multivariate analyses [23]. An explanation for these discordant results remains to be provided. However, there have been no previous reports on OGG1, MUTYH, and APEX1 with regard to survival in lung cancer. Thus, our report is the first on the detailed effects of DNA repair gene polymorphisms on the survival of Japanese lung cancer patients. In addition, numerous clinical features may play important roles in the survival of lung cancer patients. We found a significant association between survival and tumor histology. The disease stage at the time of diagnosis has a direct impact on the survival rate. Multivariate analysis showed that a higher stage (stages III and IV), male gender, older age, squamous cell carcinoma, smoking history, lymph node metastasis, and recurrence were independent prognostic factors associated with an increased risk of death. This higher

tumor stage had a significant effect on survival, which is in accordance with other studies [18,24]. It is possible that individuals with these factors and with higher-stage disease already have too many genetic alterations during their tumor growth, which would reduce their survival. Our study has several limitations, especially the fact that the conclusion was based on several patients. Our data may be biased by the relatively small number of patients as a hospital-based case–control study. Therefore, further verification of these predictive biomarkers is required with a larger study population. The gene–environment interaction between smoking and these genotypes also needs to be clarified.

4. Experimental Section

4.1. Study Subjects

Study subjects included 99 lung cancer patients (65 with lung adenocarcinoma, 29 with lung squamous cell carcinoma, and 5 with other carcinomas) who were after surgical treatment but not receiving radiotherapy and chemotherapy and were included in previous studies that investigated the genetic polymorphisms of DNA repair proteins and metabolic enzymes [7,9,25]. These patients were recruited between April 2001 and July 2002 at the Hyogo Cancer Center. Informed consent was obtained from each patient. Detailed data on smoking were obtained by personal interviews. The study design was approved by the Ethics Review Committee on Genetic and Genomic Research, Kobe University Graduate School of Medicine. All samples were coded after the collection of blood and smoking frequency data. All patients were followed up for survival by November 2009 (the time of data analysis). Metastases were based on the status of pathological metastasis.The amount of smoke exposure was calculated as pack-years; the product of the number of years an individual smoked and the average number of cigarettes smoked per day (converted into a standard pack of 20 cigarettes).

4.2. Genotyping

Genomic DNA used for this study was isolated in previous studies [7,9,25]. The genotypes of *OGG1* Ser326Cys, *MUTYH* Gln324His, *APEX1* Asp148Glu, *XRCC1* Arg399Gln, and *XRCC3* Thr241Met were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis, as described previously [7,9].

4.3. Statistical Analysis

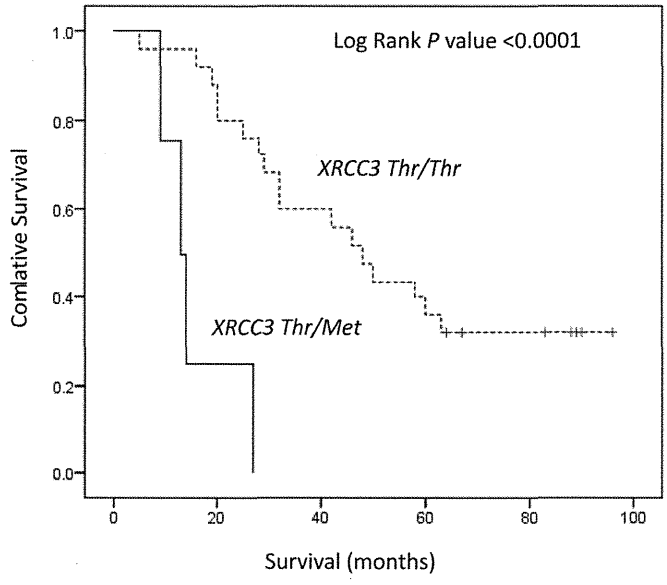
The effect of genetic polymorphisms on survival was also estimated using the Kaplan–Meier method and assessed using the log-rank test. The influence of clinical parameters on the outcomes of lung cancer patients was assessed by the log-rank test. The overall survival duration of lung cancer patients was calculated from the 1st day of treatment until either death or the last follow-up. A multiple Cox regression model was used to obtain the adjusted hazard ratio (HR) and 95% confidence interval (95% CI) for potential prognostic factors in lung cancer patients. All p values were calculated from 2-tailed statistical tests. Statistical analysis was performed with the PASW software package (version 17.0 for Windows; SPSS Japan, Inc., Tokyo, Japan). A p value of <0.05 was considered significant for an association between a genotype and lung cancer.

Table 3. DNA Repair Gene Polymorphisms and Patient Survival in relation to Subtypes.

Genotype	Patients *n*	MST (mon)	Log-rank *p* value	HR (95% CI)	Adjusted HR *p* value	Adjusted HR (95% CI) [a]	Adjusted HR *p* value
Adenocarcinoma							
OGG1							
Ser/Ser	16	62	-	1.00	-	1.00	-
Ser/Cys, Cys/Cys	49	68	0.784	0.89 (0.38–2.08)	0.785	0.80 (0.29–2.26)	0.803
MUTYH							
Gln/Gln	13	70		1.00		1.00	
Gln/His, His/His	52	66	0.65	1.25 (0.48–3.27)	0.653	1.54 (0.44–5.36)	0.498
APEX							
Asp/Asp	28	63	-	1.00	-	1.00	-
Asp/Glu, Glu/Glu	37	70	0.549	0.80 (0.39–1.67)	0.553	1.14 (0.48–2.73)	0.763
XRCC1							
Arg/Arg	26	71		1.00	-	1.00	-
Arg/Gln, Gln/Gln	39	64	0.522	1.28 (0.60–2.76)	0.526	0.87 (0.35–2.20)	0.775
XRCC3							
Thr/Thr	58	67	-	1.00	-	1.00	-
Thr/Met, Met/Met	7	64	0.995	1.00 (0.30–3.32)	0.995	1.32 (0.38–4.64)	0.661
Squamous Cell Carcinoma							
OGG1							
Ser/Ser	8	52	-	1.00	-	1.00	-
Ser/Cys, Cys/Cys	21	48	0.824	1.11 (0.43–2.88)	0.825	1.25 (0.44–3.55)	0.670
MUTYH							
Gln/Gln	5	64	-	1.00	-	1.00	-
Gln/His, His/His	24	45	0.377	1.72 (0.51–5.85)	0.385	4.73 (0.51–5.85)	0.153
APEX							
Asp/Asp	11	40		1.00	-	1.00	-
Asp/Glu, Glu/Glu	18	54	0.328	0.65 (0.27–1.56)	0.334	0.43 (0.14–1.32)	0.139
XRCC1							
Arg/Arg	16	50		1.00	-	1.00	-
Arg/Gln, Gln/Gln	13	46	0.991	1.00 (0.42–2.37)	0.991	1.17 (0.46–2.94)	0.743
XRCC3							
Thr/Thr	25	48	-	1.00	-	1.00	-
Thr/Met, Met/Met	4	13	<0.0001	9.35 (2.52–34.68)	0.001	9.05 (1.89–44.39)	0.006

a: HR adjusted for gender, age, smoking history, disease stage, metastasis, and reccurence.

Figure 1. Kaplan–Meier survival curve of lung squamous cell carcinoma patients with the *XRCC3 Thr241Met* genotype.



5. Conclusions

We analyzed the association between polymorphisms of five DNA repair genes and the outcome of Japanese lung cancer patients. Our results suggest that the *XRCC3* Thr241Met gene polymorphism plays an important role in the overall survival of Japanese lung squamous cell carcinoma patients without chemotherapy. The *XRCC3* Thr241Met gene polymorphism may be a prognostic factor in lung squamous cell carcinoma patients.

Conflict of Interest

The authors declare no conflict of interest.

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The role of Necl-5 in the invasive activity of lung adenocarcinoma

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ABSTRACT

Nectin-like molucule-5 (Necl-5) is an immunoglobulin-like molecule that was originally identified as a poliovirus receptor and is often upregulated in cancer cells. It has been said that Necl-5 plays a role in not only cell–cell adhesion, but also cell migration, proliferation, and metastasis. In this study, we used a bronchioloalveolar carcinoma (BAC) cell line and fibroblasts to assess the expression of Necl-5 in the development of cancer–stroma communication by using an easy-to-prepare double-layered collagen gel hemisphere (DL-CGH) system that enables visualization of cell migration during invasion. The expression of Necl-5 was higher in BAC cells than in fibroblasts. This tendency didn't change even when the BAC cells were mixed with fibroblasts. To assess the role of Necl-5 in the invasive activity of the BAC cells, we knocked down its expression using RNA interference (RNAi). The invasion assay with DL-CGH revealed that inhibition of Necl-5 expression in the BAC cells was associated with suppressed invasiveness. In addition, Necl-5 knockdown inhibited the movement and proliferation of the BAC cells. Necl-5 expression in lung cancer cells is crucial for their invasiveness in the cancer–stromal interaction, suggesting that Necl-5 could be a favorable molecular target for the suppression of invasiveness in lung adenocarcinoma.

Introduction

Nectins, which are Ca^{2+}-independent cell–cell adhesion molecules, are instrumental in the formation of cadherin-based adherens junctions in fibroblasts and epithelial cells (Takai et al., 2003, 2008). Recently, the role of nectin-like molecules (Necl), which have been characterized on the basis of their structural similarity to nectin and their function in cell–cell adhesion, has been clarified (Ikeda et al., 2003; Miyoshi and Takai, 2007; Takai et al., 2008). Necl-5, one of the Necl family members, is preferentially localized at the leading edge of moving cells where it promotes cell movement and proliferation (Ikeda et al., 2003; Kakunaga et al., 2004; Miyoshi and Takai, 2007; Sato et al., 2004; Takai et al., 2008). The expression of Necl-5 is downregulated when cultured cells become confluent, resulting in the suppression of cell movement and proliferation (Fujito et al., 2005). Thus, down-regulation of Necl-5 is, at least in part, one mechanism that underlies contact inhibition of cell movement and proliferation.

Necl-5 is overexpressed in various human carcinomas (Chadeneau et al., 1994; Faris et al., 1990; Koike et al., 1990; Lim et al., 1996), and experiments using transformed and cancer cell lines that express Necl-5 have shown that the downregulation of Necl-5 in these cells decreases migration, proliferation, and metastasis (Ikeda et al., 2003; Kakunaga et al., 2004; Sloan et al., 2004). We previously showed that overexpression of Necl-5 in cancer cells had clinical significance for poor prognosis in lung adenocarcinoma patients (Nakai et al., 2010). Furthermore, microscopic observation revealed that the Necl-5 was strongly stained in the invasive sites of the tumors, where stromal cells and cancer cells were mixed in a disorderly pattern.

Clinical studies demonstrated that a poor prognosis for patients with bronchioloalveolar carcinoma (BAC) depended on the presence of proliferating stromal fibroblasts (Noguchi et al., 1995). Our previous in vitro experiments showed that the invasive migration of A549 BAC cells depended on the presence of fibroblasts (Takata et al., 2007). These findings suggested that cancer–stromal interactions play an important role in the infiltration of BAC cells. Because Necl-5 was strongly expressed in the invasive front of BAC, we hypothesized that the control of Necl-5 in lung adenocarcinoma would inhibit the cancer–stromal interaction, resulting in inhibition of cancer cell migration and proliferation. In the present study, we assessed the role of Necl-5 in cancer–stromal interactions by using the simple 3-D invasion assay that we established previously (Doi et al., 2011; Takata et al., 2007).

Material and methods

Cell lines

The A549 BAC cell line was obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University; the WI-38 (lung fibroblast) cell line was obtained

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from the Health Science Research Resources Bank (Osaka, Japan); the green fluorescent protein (GFP)-labeled A549 BAC cell line was obtained from AntiCancer Corp. (Osaka, Japan). Cells were maintained in RPMI-1640 medium supplemented with penicillin (100 U/ml), streptomycin (100 U/ml), and 10% bovine calf serum (at 37 °C, 5% CO_2).

Western blotting

Cultured cells were washed with phosphate buffered saline (PBS) and then lysed with 100 μl Laemmli sample buffer, after which 10 μl of the samples were separated using SDS-PAGE. The separated proteins were then transferred to nitrocellulose membranes (GE Healthcare Corp.), which were washed with PBS-T and then blocked for 30 min with a PBS-T solution containing 5% skim milk. Blocked membranes were then rinsed twice with PBS-T and incubated (1 h, room temperature) with Necl-5 primary antibody (#ab103630; Abcam, Cambridge, UK) and focal adhesion kinase (FAK) primary antibody (#ab4803; Abcam, Cambridge, UK), which were diluted 1:500 with 5% BSA/PBS-T. FAK is a tyrosine kinase that resides at the sites of focal adhesion, which has been shown to be an important mediator of cell adhesion and migration (Golubovskaya, 2010). After the membranes were washed with PBS-T, membranes were incubated (30 min, room temperature) with the secondary peroxidase-labeled donkey anti-rabbit Ig whole antibody (GE Healthcare Corp.), which was diluted 1:5000 with PBS-T. Membranes were washed with PBS-T and then treated with a chemiluminescent detection kit (GE Healthcare Corp.) before they were visualized using a luminoimage analyzer (LAS-3000; Fujifilm Inc., Tokyo, Japan).

As a control assay, immunoblotting was performed on the same membranes with a primary antibody directed against β-actin (#4967, Cell Signaling Technology), followed by a peroxidase-labeled donkey anti-rabbit Ig whole secondary antibody (GE Healthcare Corp.).

Preparation of a double-layered collagen gel hemisphere (DL-CGH)

Acid-soluble collagen 1 (Nitta Gelatin Inc., Osaka, Japan), 10-fold concentrated Ham's F-12 medium, and reconstruction buffer (2.2 g $NaHCO_3$ + 4.77 g HEPES in 100 ml of 0.05-N NaOH) were mixed in a volume ratio of 8:1:1 and then seeded with cells cultured at a density of 3.0×10^6 cells/ml. Five microliters of the mixture containing 3.0×10^4 cells was then dropped onto a plastic dish. Once the mixture had gelled, a second 30-μl drop of collagen was placed directly on the top of the first gel drop, encapsulating it completely. The gel hemisphere was then submerged in medium and cultured.

RNA interference (RNAi) in the A549 BAC cell lines

RNAi was performed with commercially available siRNA for Necl-5 (target sequence, CAG GCT ATA ATT GAA GCA CGA; Qiagen GmbH) and a non-silencing control siRNA (AllStars Negative Control siRNA; Qiagen GmbH) according to the manufacturer's instructions. Briefly, 20 μl of transfection reagent (Hiperfect; Qiagen GmbH) was suspended in 200 μl of serum-free culture medium containing 2.5 μM siRNA. After a 10-min incubation at room temperature, the mixture was added to A549s (60-mm round dish with 4 ml culture medium containing 10% fetal bovine serum and antibiotics mentioned above) grown to 80% confluence; the final concentration of the siRNA was 100 nM. After 24 h (at 37 °C, 5% CO_2), these cells were suspended in PBS, and the cell density was calculated to prepare for the encapsulation of the cells in DL-CGH.

Evaluation of A549 and WI-38 cell invasion

To observe the activity of Necl-5 in the interaction between GFP-labeled A549s and WI-38s, 1.5×10^4 cells of each type were mixed in the first gel layer and incubated for 4 days. The invasion activity of the cells was then evaluated by counting the GFP-labeled A549s in the outer collagen layer by using a BZ9000 fluorescence microscope (Keyence Corp., Tokyo, Japan). In separate experiments, GFP-labeled A549s were first transfected with either inhibitory RNA for Necl-5 or non-silencing control siRNA before they were embedded in the first gel layer. The invasion activity of the transfected cells was then compared.

Time-lapse motion picture of the cell movement

A BZ9000 fluorescence microscope with a CO_2 chamber was used to evaluate cell movement. DL-CGH prepared on a 35-mm round plastic dish with 2 ml medium was mounted on the microscope-table incubation chamber, and a 10× objective image was displayed on the monitor. Recording started at 48 h after DL-CGH culture initiation, and continued for 12 h. The movement of the GFP-labeled A549s was then calculated. In addition, 20× fluorescent images of the GFP-labeled A549 were captured every hour from 48 h after culture initiation to 168 h, and stored as 1360×1024-pixel TIFF files. A movie (saved as an MPEG-2 file) was then created, which displayed 120 consecutive images, with 0.2 s per image, by using the EDIUS ver.5.0 software (Grass Valley K.K., Kobe, Japan).

Statistical analysis

Statistical significance was determined using unpaired Student's *t*-test. All tests were performed with JMP ver. 8.0 statistical software (SAS Japan Institute Inc., Tokyo, Japan).

Results

Necl-5 expression in cancer cells and normal fibroblasts

Western blotting was performed to determine whether Necl-5 and FAK were expressed in the cell lines of bronchioloalveolar carcinoma (A549), fibroblast (WI-38), and a mixture of each cell type. We detected strong expression of Necl-5 in the A549 cell line. However, the expression of Necl-5 was very weak in WI-38s. In the mixture of A549 and WI-38, Necl-5 was moderately expressed. FAK expression showed the same tendency of Necl-5 (Fig. 1). These findings suggest that both Necl-5 and FAK are strongly expressed in A549s in both the absence and presence of WI-38s.

Effect of RNAi-mediated knockdown of Necl-5 on the invasive activity of cancer cells

We previously showed that A549 cells, which were unable to infiltrate the outer layer alone, opportunistically invade along the paths of infiltrating WI-38s in the DL-CGH test. We therefore decided to use this assay to assess if the invasive activity of A549 cell lines is controlled by Necl-5 by inhibiting expression of Necl-5 in A549s. Initial knockdown studies showed that cells transfected with Necl-5 siRNA have a 50% reduction in the expression of both Necl-5 and FAK protein relative to cells transfected with control siRNA (Fig. 2). In the DL-HCG test, GFP-labeled A549 transfected with Necl-5 RNAi and GFP-labeled A549 transfected with control non-silencing siRNA were mixed with WI-38 in the inner layer. After 4 days of incubation, invasive activity (represented by the number of GFP-labeled A549 in the outer collagen layer) was measured. The number of A549 cells in the group transfected with Necl-5 RNAi showed a significant decrease relative to the number in the non-treated group or the group transfected with control non-silencing siRNA (Figs. 3 and 4). Thus, inhibition of Necl-5 in A549 resulted in suppressed invasive activity.

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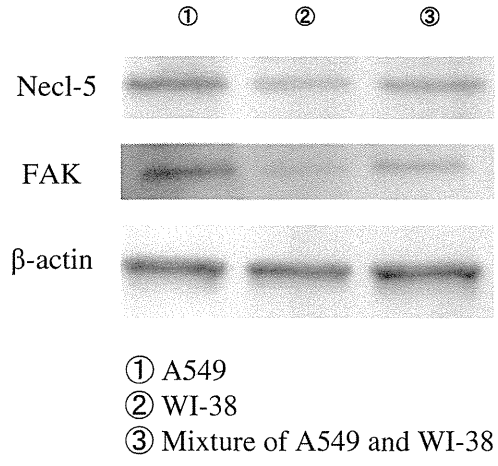

Fig. 1. *Western blotting for Necl-5, FAK and β-actin.* Expression of Necl-5 was stronger in A549 cells than in WI-38 cells. In the mixture of A549s and WI-38s, Necl-5 was moderately expressed. FAK expression showed the same tendency of Necl-5.

Effect of RNAi-mediated knockdown of Necl-5 on cell proliferation

Since it is difficult to evaluate proliferation using the DL-HCG model, we assessed the proliferative effects of Necl-5 on A549s on normal tissue culture plates (60-mm-round dish with 4-ml culture medium containing 10% fetal bovine serum and antibiotics as mentioned above) containing 40×10^4 A549s cells transfected with either Necl-5 RNAi or control non-silencing siRNA. The number of cells in each transfected cell line was counted every 24 h from culture initiation up until 72 h. The cells transfected with Necl-5 RNAi grew more slowly when compared to the other cell lines (Fig. 5), which reveals that the knockdown of Necl-5 inhibits proliferation of A549 cells.

Effect of RNAi-mediated knockdown of Necl-5 on cell movement

The cell movement of GFP-labeled Necl-5 transfected A549s was also evaluated, because invasiveness in the DL-CGH models might be affected by rates of proliferation. As noted above, we created 3 types of DL-CGH models (GFP-labeled A549, A549s transfected with Necl-5 RNAi, and A549s transfected with control non-silencing siRNA, each mixed with fibroblasts in the inner layer). In this study, we then observed GFP-labeled A549 movement over 12 h by using the time-lapse method and compared the migration distance in each models (Fig. 6a). Compared to other models, these models revealed that A549s transfected with Necl-5 RNAi moved more slowly along the surfaces of the fibroblasts (Fig. 6b).

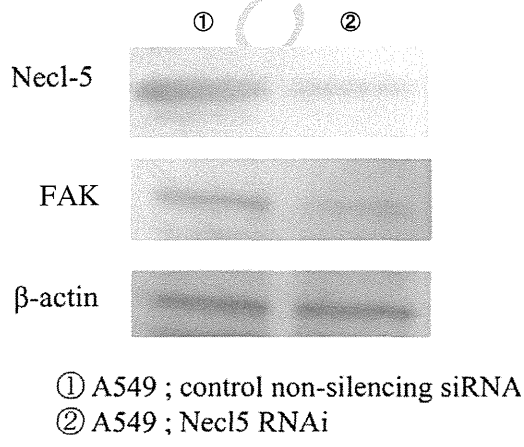

Fig. 2. *Western blotting after transfection with Necl-5 RNAi.* Immunoblotting showed a 50% reduction in the expression of both Necl-5 and FAK proteins relative to cells transfected with control non-silencing siRNA.

Discussion

Necl-5, which was previously named Tage4, plays a role not only in cell–cell adhesion, but also in cell migration, proliferation, and metastasis (Ikeda et al., 2003; Kakunaga et al., 2004; Miyoshi and Takai, 2007; Morimoto et al., 2008; Sato et al., 2004; Takai et al., 2008). Necl-5 colocalizes with integrin αvβ3 at the leading edges of moving cells (Ikeda et al., 2004), and through activation of Cdc42 and Rac, induces the formation of filopodia and lamellipodia, which eventually enhance growth factor-induced cell movement (Takai et al., 2001). In addition, Necl-5 also enhances the proliferation induced by growth factor receptors, such as PDGF and FGF (Kakunaga et al., 2004), and through activation of the Ras–Raf–MEK–ERK signaling, causes upregulation or downregulation of the cell cycle regulators, including cyclins D2, E, and p27kip1. These effects contribute to shortening of the G1 phase of the cell cycle and enhancement of growth factor-induced cell proliferation. Necl-5 does not show any homophilic cell–cell adhesion activity, but does heterophilically trans-interact with nectin-3 (Ikeda et al., 2003). When cells come into contact with other cells, Necl-5 is downregulated from the cell surface by its trans-interaction with nectin-3, thereby leading to inhibition of cell migration and proliferation (Fujito et al., 2005; Sato et al., 2004). Transformation of cells, which induces high levels of Necl-5 expression, causes disruption of cell–cell adhesion, increase of cell motility, and loss of contact inhibition of cell movement and proliferation, eventually leading to cancer invasion.

Necl-5 is highly expressed in various cancer cell lines and human cancer tissues (Chadeneau et al., 1994; Faris et al., 1990; Ikeda et al., 2003; Koike et al., 1990; Lim et al., 1996; Nakai et al., 2010; Sloan et al., 2004). We previously reported that the abnormal overexpression of Necl-5 in cancer cells has negative prognostic factors for primary lung adenocarcinomas (Nakai et al., 2010). According to this report, the microscopic observation of immunostaining revealed that Necl-5 was strongly expressed especially in the peripheral area of the BAC, where cancer cells infiltrated aggressively into the stroma, but not at the tumor center in many of the BACs. Tokunou et al. have reported the importance of cancer–stromal communication for the development of the invasive component of BAC (Tokunou et al, 2001). We assumed that such overexpression of Necl-5 might play a role in the aberrant behavior at the invasive front of BAC, and set out to assess the activity of Necl-5 in the development of cancer–stromal communication by in vitro analysis. In this study, we revealed that inhibition of Necl-5 in cancer cells reduced their invasiveness and proliferation. However, Necl-5 physiologically suppresses cell movement and proliferation in normal cell–cell contact (Fujito et al., 2005). It is still not clear why this normal function of Necl-5 was lost in the cancer–stromal interaction.

Cell migration is a complex so-called "adhesion turnover" process that involves the continuous formation and disassembly of adhesions. Adhesion formation takes place at the leading edge of cell protrusions, whereas disassembly occurs both at the cell near and at the base of protrusion. FAK is known to be crucial for adhesion turnover at the cell front, a process central to migration. Normal tissues have low expression of FAK, while cancer cells significantly overexpress this protein (Golubovskaya, 2010; Webb et al., 2004). In this study, both Necl-5 and FAK are strongly expressed in A549s in both the absence and presence of WI-38s. Moreover, A549s transfected with Necl-5 siRNA have a 50% reduction in the expression of both Necl-5 and FAK proteins. These results supported that the overexpression of Necl-5 in cancer cells might be related to FAK in cancer cell migration.

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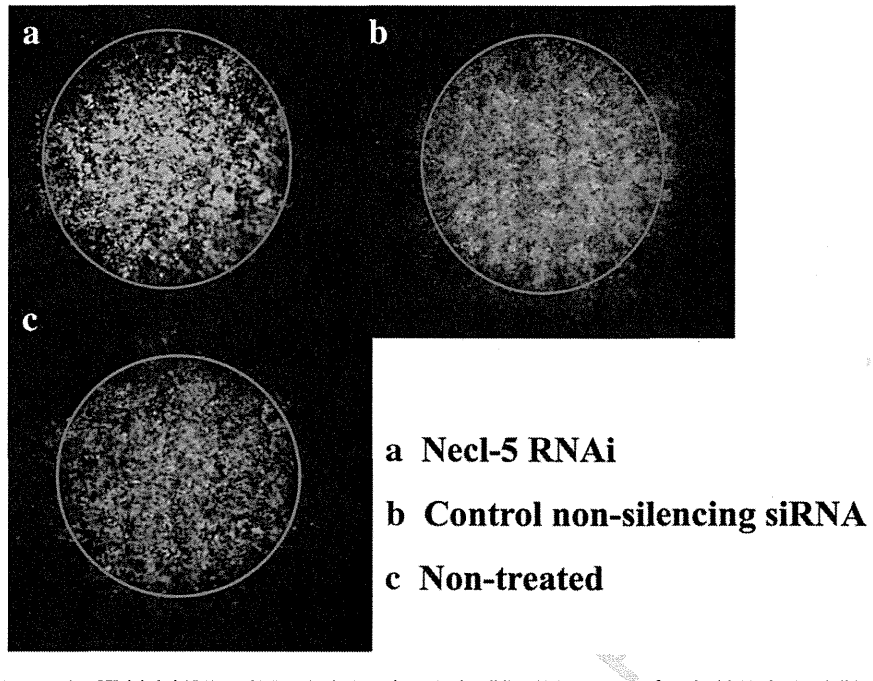

Fig. 3. *Results from the DL-CGH assay using GFP-labeled A549s and WI-38 in the inner layer.* Each cell line ((a) A549s transfected with Necl-5 RNAi, (b) A549s transfected with control non-silencing siRNA, and (c) non-treated A549s) was mixed with WI-38s in the inner layer (blue line). A549s transfected with Necl-5 RNAi showed less invasiveness into the outer layer relative to the other cell lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The differences among the different tissue types of human lung adenocarcinoma, such as BAC, papillary, acinar, and solid carcinomas, remained controversial in clinical practice (Kerr, 2009). Human lung adenocarcinomas are often composed of mixed components of these tissue types. Noguchi et al. (1995) reported that patients with BAC containing actively proliferating fibroblasts show a worse prognosis than do patients with small-sized BAC, in which cancer cells spread on the internal surface of alveoli but do not infiltrate interstitially. This result was consistent with the fact that the abnormal over-expression of Necl-5 in the invasive front of cancer cells in primary lung adenocarcinomas negatively affects the prognosis of patients.

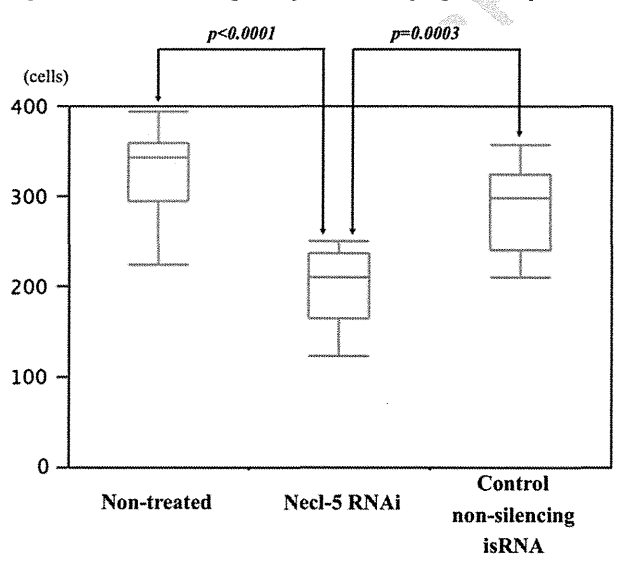

Fig. 4. *Comparison of A549 invasive activity by using the DL-CGH assay (represented by the number of GFP-labeled A549s in the outer collagen layer).* A549 cells transfected with Necl-5 RNAi showed a significant decrease in invasive activity relative to the non-treated group ($p<0.0001$) or control non-silencing siRNA transfected group ($p=0.0003$) ($n=10$ samples for each group).

Many studies have shown that disruption of cell–cell adhesion of tumor epithelial cells, resulting in epithelial–mesenchymal transition (EMT), causes tumor progression and is highly related to poor prognosis for cancer patients (Bellovin et al., 2005; Shioiri et al., 2006; Soltermann et al., 2008; Thiery, 2002). Liotta et al. demonstrated Q5 that cancer invasion occurs within a tumor–host microecology, where stroma and tumor cells exchange enzymes and cytokines that modify the local extracellular matrix, stimulate migration, and promote proliferation and survival (Liotta and Kohn, 2001). Thus, the presence of fibroblasts is essential for cancer invasion. In fact, we showed that A549s (BAC) in the inner layer of the DL-CGH model could not infiltrate into the outer layer by themselves, whereas A549 mixed with WI-38 (fibroblast) widely spread into the outer layer (data not shown). Moreover, the A549 BAC cells, like amoebas, move and spread along the fibroblasts (Takata et al., 2007). This fact

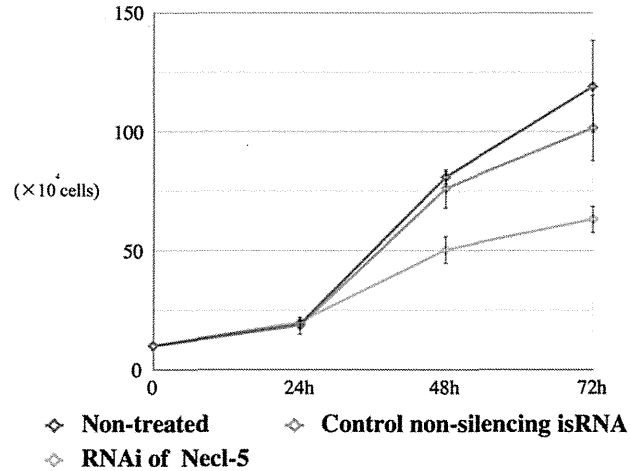

Fig. 5. *Comparison of Necl-5-transfected A549 proliferative activity.* A549 cells transfected with Necl-5 RNAi grew slower compared to normal cells and control-treated cells ($n=5$ samples for each group).

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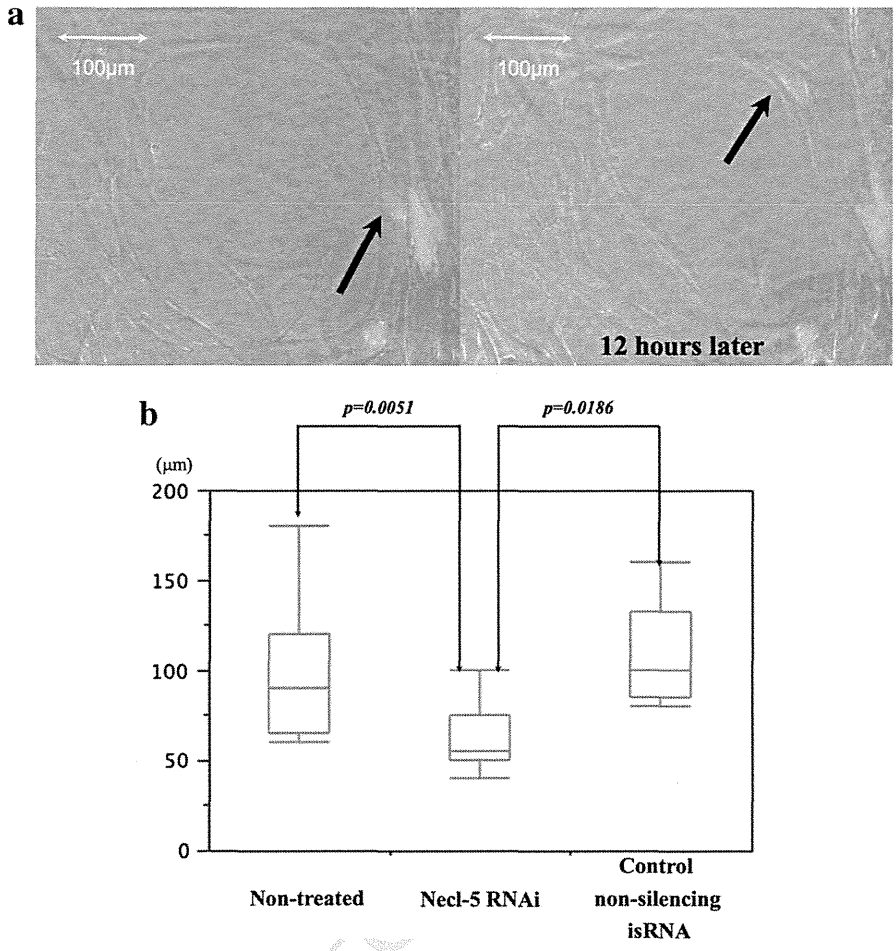

Fig. 6. *Comparison of Necl-5-transfected A549 cell movement.* (a) Neutral and fluorescent images (×100) of GFP-labeled A549s captured separately and then merged. From 48 h after the initiation of culture to 60 h, the distance of GFP-labeled A549 cells (marked with yellow arrows), which moved approximately 100 μm along the surface of fibroblasts, was calculated. (b) The group of A549 transfected with Necl-5 RNAi moved significantly slower compared to the non-treated group ($p = 0.0051$) and the group transfected with control non-silencing ($p = 0.0186$) ($n = 10$ samples for each group). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that the cell shape does change during amoeba-like movement involving the cytoskeleton might reflect the EMT.

In this study, compared to fibroblasts, the BAC cells showed a strong expression of Necl-5. The tendency did not change even if the BAC cells were mixed with fibroblasts. The DL-CGH method also revealed that the inhibition of Necl-5 in tumor cells suppressed cell migration. This finding indicates that the expression of Necl-5 that originated in cancer cells might be crucial for the cancer–stromal communication, eventually leading to EMT. Therefore, Necl-5 could be a suitable molecular target for the suppression of cancer invasion in future studies.

We have previously shown the utility of DL-CGH, which enabled precise visualization of the invasive activity of cells precisely (Takata et al., 2007). Using this method, it would be feasible to conduct other large-scale RNAi experiments to screen for molecular gene targets against cancer infiltration. In contrast, it is difficult to assess cell proliferation by using this method. With regard to cell proliferation, we evaluated A549 cells only in cell culture dishes, and did not assess proliferation in the relation to fibroblasts. Further studies should be performed before the proliferative effects of Necl-5 on cancer–stromal interactions can be fully understood.

In conclusion, the expression of Necl-5 in tumor cells is associated with cell movement and proliferation. Furthermore, the DL-CGH method revealed that the knockdown of Necl-5 inhibited cancer invasiveness in cancer–stromal interactions. Necl-5 expression in tumor cells may play a crucial role in cancer–stromal communication and may be a potential molecular target for lung adenocarcinoma.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yexmp.2012.12.003.

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INSTITUTIONAL REPORT

Radical hybrid video-assisted thoracic segmentectomy: long-term results of minimally invasive anatomical sublobar resection for treating lung cancer

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Abstract

We analysed the results of radical segmentectomy achieved through a hybrid video-assisted thoracic surgery (VATS) approach that used both direct vision and television monitor visualization at a median follow-up of over 5 years. Between April 2004 and October 2010, 102 consecutive patients able to tolerate lobectomy to treat clinical T1N0M0 non-small cell lung cancer (NSCLC) underwent hybrid VATS segmentectomy in which we used electrocautery without a stapler to divide the intersegmental plane detected by selective jet ventilation in addition to the path of the intersegmental veins. Curative resection was achieved in all patients. The median surgical duration and blood loss during the surgery were 129 min (range, 60–275 min) and 50 ml (range, 10–350 ml), respectively. The complication rate was 9.8% (10/102) with the most frequent being prolonged air leak, and there was no case of in-hospital death or 30-day mortality post procedure. Five and seven patients developed locoregional and distant recurrences, respectively. The overall and disease-free 5-year survival rates were 89.8% and 84.7%, respectively. Radical hybrid VATS segmentectomy including atypical resection of (sub)segments is a useful option for clinical stage-I NSCLC. The exact identification of anatomical intersegmental plane followed by dissection using electrocautery is critical from oncological and functional perspectives.

Keywords: Segmentectomy • Lung cancer • Video-assisted thoracic surgery • Sublobar resection

INTRODUCTION

Advances in radiographic devices such as high-resolution computed tomography (CT) and the widespread practice of low-dose helical CT for screening have resulted in an extraordinary increase in the early detection of ever smaller non-small cell lung cancers (NSCLCs), such as bronchioloalveolar carcinoma, that might possibly have more indolent biological behaviour. This trend has rapidly changed clinical practice in thoracic surgery, and thus concern has arisen over unified strategies that include whole lobectomy to treat small peripheral cancers. Removing a relatively large volume of healthy lung tissue could result in a poorer quality of postoperative life, a higher frequency of operative morbidity and a decreased likelihood of having a second or even a third NSCLC resected, for which such patients would survive long enough to become at risk. We have therefore actively performed radical segmentectomy with lymph node assessment not only for high-risk but also for good-risk patients with small clinical stage-I NSCLC [1–4]. The outcomes of the randomized study conducted by the Lung Cancer Study Group demonstrated that sublobar resections including wedge resections resulted in a higher rate of local recurrence compared with lobectomy in patients with clinical T1N0M0 NSCLC [5]. Thus, the incidence of non-anatomical stapled wedge resection has escalated and many recent residency programs in thoracic surgery do not cover segmentectomy as a mandatory procedure. However, we and other expert surgeons perceive segmentectomy as a crucial basic technique that should be mastered by all thoracic surgeons [6, 7].

We previously reported a novel segmentectomy in which the intersegmental plane was identified using selective jet ventilation under bronchofiberscopy [8]. Consequently, the segment to be removed can be inflated, whereas those to be preserved are maintained while deflated, which is contrary to the conventional procedure. However, this allows clear visualization of the anatomical intersegmental line between the segment to be resected and that to be preserved. The actual surgical margin in the inflated segment can be adequately grasped and a good surgical field can be obtained even through video-assisted thoracic surgery (VATS) without the need to physically suppress the other segments and lobes using an instrument. In addition, the anatomical intersegmental plane can be precisely dissected by electrocautery without any stapling. This allows the saved adjacent segments to remain completely expansive so that pulmonary function after surgery can be maximal.

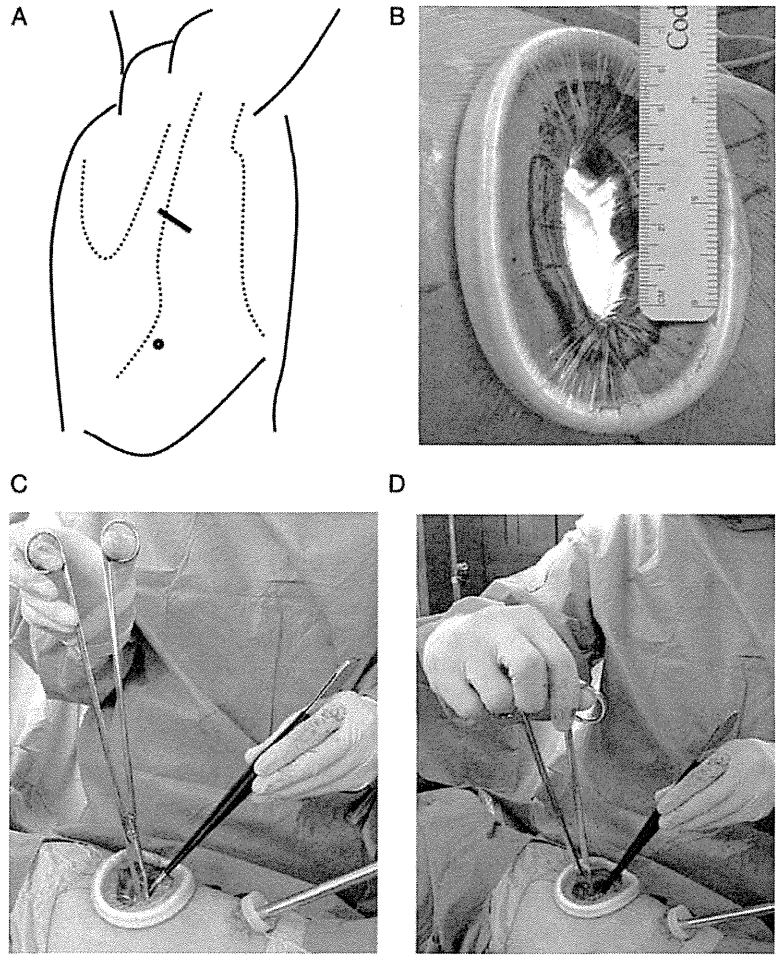

Figure 1: Hybrid VATS approach. (**A**) Skin is incised for one access port for a thoracoscope (circle) and for an access thoracotomy (solid line) over the mid-axillary line in the fourth interspace for upper or middle lobe tumours. Lower lobe tumours are approached through auscultatory triangle in the fifth interspace. (**B**) Operative exposure of about 4-cm wide is achieved using a wound retractor without rib spreading. (**C** and **D**) Sharp dissection in depths of an open thorax through direct vision is performed using an upside-down grip on 30-cm scissors, which allows manipulation with the thumb and index finger through the loops and flexible manoeuvring by turning up the wrist. The ulnar side of the hands rest comfortably alongside the margins of the incision, and awkward elevation of the forearms or elbows can be avoidable.

Basically, complete VATS using only a monitor for visualization is limited to lung resections of minimal difficulty and cannot be applied to all cancer surgeries, including segmentectomy and bronchoplasty. We have applied hybrid VATS, an integrated surgical approach that combines a muscle-sparing minithoracotomy and a thoracoscopic hole with television monitoring and direct visualization to expand the use of minimally invasive techniques for treating various malignant pathologies [9]. The present study analyses the surgical results of radical hybrid VATS segmentectomy at a median postoperative follow-up of over 5 years.

METHODS

Hybrid VATS segmentectomy

The hybrid VATS approach generally requires two skin incisions for access without cutting muscles or ribs [9]. One 4–5-cm incision is for manipulation and the other is an access port of 1-cm long for insertion of a thoracoscope (Fig. 1). The surgeon directly observes the hilum of the diseased lobe through the main access using a silicon rubber wound retractor with no rib spreading, and then individually isolates and severs all bronchi and vessels of the segment in question utilizing television monitor guidance when dissecting an area that is out of direct view. The skin incision was immediately extended when the surgeon has difficulty with the surgical view. Avoiding rib spreading is critical to the definition of a VATS approach. If the ribs were spread or a skin incision of ≥8 cm long was required, then the patient was excluded from the present study because it was considered a conversion to thoracotomy. We prefer a back hand grip to hold 30-cm-long scissors (model 101-8098-30; Mayo-Harrington; Stille, Sweden) for sharp dissection, long needle holders and forceps upside down (Fig. 1). Immediately after the insertion of a thoracoscope, subclinical dissemination of the tumour was checked by pleural lavage cytology [10].

The procedure of radical segmentectomy has been outlined in a previously published article [8]. Identifying the intersegmental plane with jet ventilation and cutting the intersegmental parenchyma using cautery are unique features of the procedure.