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Original Article

An immunohistochemical and molecular genetic analysis of *KIT* and *PDGFRA* in small cell lung carcinoma in Japanese

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Abstract: KIT and PDGFRA in small cell lung carcinoma (SCLC) have been rarely examined in Japanese. The author investigated protein expression of KIT and PDGFRA in 54 Japanese cases of small cell lung carcinoma by immunohistochemistry, and gene mutations of *KIT* and *PDGFRA* in 20 Japanese cases of small cell lung carcinoma by the PCR-direct sequencing method. The molecular genetic analysis showed no mutations of *KIT* (exons 9, 11, 13, and 17) and *PDGFRA* (exons 12 and 18) genes in all 20 cases. KIT protein expression was recognized in all cases (100%). Membranous KIT expression was strong in 35 cases, moderate in 7 cases and weak in 12 cases. PDGFRA protein expression was noted in 35 cases (65%); the membranous expression was strong in 2 cases, moderate in 16 cases, and weak in 17 cases. The overall median survival was 13 months. There was no significant difference in the survival between KIT strongly positive cases (median, 12 months) and KIT moderately or weakly positive cases (median, 11 months). Likewise, there was no significant difference in the survival between PDGFRA-positive cases (median, 11 months) and PDGFRA-negative cases (median, 12 months). The protein expressions of KIT and PDGFRA did not correlate with gender, smoking, and disease stage. These findings suggest, in Japanese population, that mutations of *KIT* and *PDGFRA* were absent in small cell lung carcinoma of Japan, that KIT protein expression is present in 100%, that PDGFRA expression is present in 65%, and that KIT and PDGFRA protein expressions do not correlate with survival, gender, smoking, and disease stage.

Keywords: Small cell lung carcinoma, *KIT*, *PDGFRA*

Introduction

Small cell carcinoma can occur in any organ, but the great majority develops in the lung. Small cell lung carcinoma (SCLC) shows aggressive behavior and poor prognosis. Recent studies of SCLC have shown that KIT protein is expressed in 30-100% of SCLC [1-10]. The difference of the percentage may be due to different primary antibodies used, staining methods, incubation periods, and interpretation of the immunostaining. In addition, one report showed that a few *KIT* gene mutations were present in SCLC [8], but others did not [7, 9, 10]. Platelet-derived growth factor receptor-α (PDGFRA) protein in SCLC has not been reported. *PDFFRA* gene in SCLC has been investigated in only one study [10], which showed no mutations in 31 cases of SCLC. However, such studies have not been performed in the yellow race including Japanese.

KIT and *PDGFRA* genes, both mapped to 4q12, encode receptor tyrosine kinase oncoprotein called KIT (CD117) and PDGFRA, respectively [11-16]. Both molecules are transmembranous oncoprotein involved in tumorigenesis of some neoplasms including gastrointestinal stromal tumor (GIST), acute myeloid leukemia, mast cell neoplasms, germ cell tumors, melanoma, neuroendocrine carcinomas, large cell neuroendocrine carcinoma and SCLC [11-16]. The hot spots of gene mutations are exons 9, 11, 13, and 17 of *KIT* gene and exons 12 and 18 of *PDGFRA* gene [11-16].

The author retrospectively investigated the protein expression of KIT and PDGFRA, gene muta-

tions of *KIT* and *PDGFRA*, and implications of clinical survival in 54 Japanese cases with SCLC.

Materials and methods

The author retrieved 54 consecutive lung biopsy samples (recent 20 years) of SCLC of 54 Japanese patients in our hospital. The mean age of the patients was 63.4±11.4 years (**Table 1**). Of the 54 cases, 52 were dead and 2 are alive (**Table 1**). Male to female ratio was 52:2. Smokers were 46 cases (85%). The 54 patients with SCLC accounted for almost 100% of patients with SCLC who underwent lung biopsy. All the 54 patients were treated in our hospital with cisplatin-based chemotherapy and radiation.

The samples had been embedded in paraffin blocks. Several 3-μm sections were cut, and one of them was stained with hematoxylin and eosin. The remaining sections were immunohistochemically examined, using the Dako's Envision method as previously reported [17-20], for KIT (polyclonal, dilution=1:100, Dako, Glostrup, Denmark) and PDGFRA (polyclonal, 1:100, Santa Cruz, CA, USA). The incubation period of each primary antibody was overnight. The diagnosis of SCLC was also confirmed by immunohistochemical staining for neuron-specific enolase (clone BBS/NC/VI-H14, 1:200, Dako), chromogranin (clone DAK-A3, 1:200, Dako), synaptophysin (polyclonal, 1:200, Dako), and CD56 (cone UJ13A, 1:150, Dako). The immunohistochemical scoring was performed according to the current scoring system of HER2/neu of breast cancer [21]. That is, in cases of positive cells < 10% was negative, and those > 10% was positive. In the positive cases, the scoring was performed according the strength of the immunostaining. Therefore, the immunohistochemical staining patterns of KIT and PDGFRA were categorized as follows: strong membranous expression 3+ (**Figure 1A** for KIT, and **Figure 2A** for PDGFRA), moderate membranous expression 2+ (**Figure 1B** for KIT, and **Figure 2B** for PDGFRA), weak membranous expression 1+ (**Figure 1C** for KIT, and **Figure 2C** for PDGFRA). Cases with positive cells < 10% was labeled as - (negative). Five cases of gastric GIST and five cases of uterine leiomyoma were employed as positive and negative controls, respectively.

A molecular genetic analysis of *KIT* gene (exons 9, 11, 13, and 17) and *PDGFRA* gene (exons 12 and 18) was performed in 20 cases by the PCR direct sequencing method, as previously reported [22-35]. The exons of both genes were selected because they are frequent mutation sites [11-16]. The primers are shown in **Table 2**. In brief, genomic DNA was extracted from paraffin sections with proteinase K digestion and phenol/chloroform extraction, and subjected to

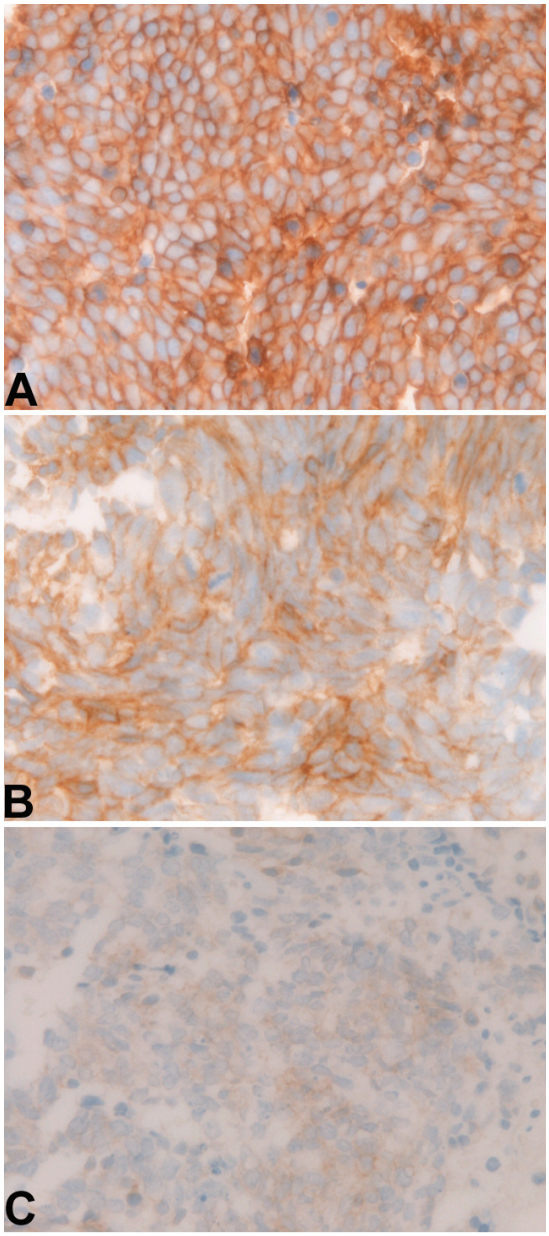


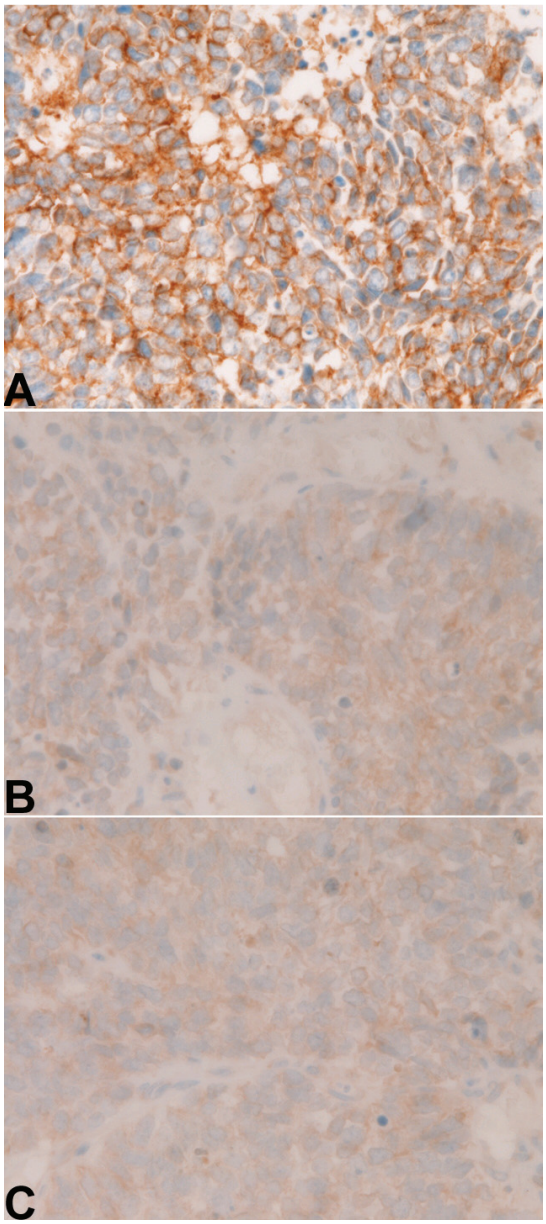
Figure 1. Expression of KIT. A: Strong membranous expression of KIT in small cell lung carcinoma. Immunostaining, X400. B: Moderate membranous expression of KIT in small cell lung carcinoma. Immunostaining, X400. C: Weak membranous expression of KIT in small cell lung carcinoma. Immunostaining, X400.

Table 1. Results of immunohistochemistry and patients' survival, smoking, and stage

Case No.	Age (yrs)	Gender	KIT	PDGFRA	Survival (months)	Smoking	TNM	Stage
1	49	male	+3	+2	14 mo	+	T2N1M0	II
2	52	male	+3	+2	11 mo	+	T3N1Mx	III
3	75	male	+3	+2	7 mo	+	T2M0N0	I
4	64	male	+3	+2	11 mo	+	T2N2M1	IV
5	68	male	+3	+2	14 mo	+	T1NxM1	IV
6	62	male	+3	+2	7 mo	+	T3N2M1	IV
7	46	male	+3	+2	9 mo	+	T2NxM1	IV
8	79	male	+3	+2	8 mo	+	T2N1M1	IV
9	63	male	+3	+1	14 mo	+	T2N0M0	I
10	71	male	+3	+1	12 mo	+	T2N0M0	I
11	68	male	+3	+1	9 mo	-	T3N2M0	III
12	51	male	+3	+1	21 mo	+	T1N0M0	I
13	67	male	+3	+1	9 mo	+	T2N0M0	I
14	82	male	+3	+1	alive (5mo)	+	T2N1Mx	II
15	53	male	+3	+1	21 mo	+	T1N0M0	I
16	49	male	+3	+1	12 mo	+	T2NxM1	IV
17	65	male	+3	+1	15 mo	+	T2N2M0	III
18	71	male	+3	+1	4 mo	-	T4N3M1	IV
19	60	male	+3	+1	15 mo	+	T2N0M0	I
20	77	male	+3	+1	19 mo	+	T1N0M0	I
21	82	male	+3	+1	6 mo	+	T3N2Mx	III
22	81	female	+3	+1	8 mo	-	T2N1M0	II
23	57	male	+3	+1	14 mo	+	T1N1M0	II
24	67	male	+3	+1	17 mo	+	T1N0M0	I
25	73	male	+3	+1	8 mo	+	T2N0M0	I
26	59	male	+3	-	11 mo	+	T2N0M0	I
27	78	female	+3	-	12 mo	-	T2N0M0	I
28	56	male	+3	-	12 mo	+	T2N1Mx	II
29	68	male	+3	-	17 mo	+	T1N0M0	I
30	55	male	+3	-	26 mo	-	T1N0M0	I
31	62	male	+3	-	10 mo	+	T2N1M1	IV
32	63	male	+3	-	alive (7mo)	+	T2N1M0	II
33	66	male	+3	-	3 mo	+	T4N3M1	IV
34	65	male	+3	-	12 mo	+	T2N0Mx	I
35	71	male	+3	-	16 mo	+	T2N0M0	I
36	64	male	+2	+3	21 mo	+	T1N0M0	I
37	52	male	+2	+3	11 mo	+	T2NxMx	I
38	75	male	+2	+2	13 mo	+	T2N0M0	I
39	81	male	+2	+2	8 mo	+	T4NxM1	IV
40	59	male	+2	+2	17 mo	-	T2N0M0	I
41	35	male	+2	+2	9 mo	+	T2NoM0	I
42	43	male	+2	+2	8 mo	+	T3N2M1	IV
43	73	male	+1	+2	17 mo	-	T2N0M0	I
44	49	male	+1	+2	16 mo	+	T2N1M0	II
45	39	male	+1	+2	6 mo	+	T3N2M1	IV
46	59	male	+1	-	15 mo	+	T1N0M0	I
47	75	male	+1	-	7 mo	+	T3N2M1	IV
48	48	male	+1	-	8 mo	+	T2N1M0	II
49	65	male	+1	-	13 mo	+	T1N0M0	I
50	79	male	+1	-	3 mo	+	T4N3M1	IV
51	64	male	+1	-	26 mo	-	T1N0M0	I
52	73	male	+1	-	15 mo	+	T2N0M0	I
53	62	male	+1	-	6 mo	+	T3N1M1	IV
54	55	male	+1	-	12 mo	+	T2N0M0	I

Table 2. Primer sequence

Forward	Reverse
KIT exon 9	
5'-TCC TAG AGT AAG CCA GGG CTT-3'	5'-TGG TAG ACA GAG CCT AAA CAT CC-3'
KIT exon11	
5'-GAT CTA TTT TTC CCT TTC TC-3'	5'AGC CCC TGT TTC ATA CTG AC-3'
KIT exon 13	
5'-GCT TGA CAT CAG TTT GCC AG -3'	5'-AAA GGC AGC TTG GAC ACG GCT TTA-3'
KIT exon 17	
5'-CTC CTC CAA CCT AAT AGT GT-3'	5'-GTC AAG CAG AGA ATG GGT AC-3'
PDGFRA exon12	
5'-TTG GAT ATT CAC CAG TTA CCT GTC-3'	5'-CAA GGG AAA AGC TCT TGG-3'
PDGFRA exon 18	
5'-ACC ATG GAT CAG CCA GTC TT-3'	5'-TGA AGG AGG ATG AGC CTG ACC-3'



PCR for 40 cycles (94°C for one minute, 52°C for one minute, 72°C for one minute), using a thermal cycler (GeneAmp PCR system 9700, Applied Biosystems, ABI, CA). The annealing temperature was 53°C. PCR products were extracted, and subjected to a computed automatic DNA sequencer (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems, ABI, CA). Two cases of gastric GIST and two cases of uterine leiomyoma were used as positive controls and negative controls, respectively.

Results

No mutations of *KIT* and *PDGFRA* genes were recognized in all the 20 cases of SCLC. The two GISTs used as positive controls showed two point mutations of *KIT* gene. The two uterine leiomyomas used as negative controls showed no mutations of *KIT* and *PDGFRA* genes.

Immunohistochemically, KIT protein expression (**Figure 1A**, **1B** and **1C**) was recognized in all the 54 cases (100%) (**Table 1**). Strong KIT expression (+3) (**Figure 1A**) was present in 35 cases (65%), moderate expression (2+) (**Figure 1B**) in 7 cases (13%), and weak expression (+1) (**Figure 1C**) in 12 cases (22%) (**Table 1**). PDGFRA expression (**Figure 2A**, **2B** and **2C**) was recognized in 35 cases (65%); strong expression (+3) (**Figure 2A**) in 2 cases (4%), moderate expression (+2) (**Figure 2B**) in 16 cases (30%),

Figure 2. Expression of platelet-derived growth factor receptor -α (PDGFRA). A: Strong membranous expression of PDGFRA in small cell lung carcinoma. Immunostaining, X400. B: Moderate membranous expression of PDGFRA in small cell lung carcinoma. Immunostaining, X400. C: Weak membranous expression of PDGFRA in small cell lung carcinoma. Immunostaining, X400

Table 3. Means and standard deviations of KIT and PDGFRA scores between smokers and non-smokers, between female and male, and between stages I+II and stages III+IV

	KIT score	PDGFRA score
Non-smoker (n=8)	2.375±0.875	1.000±0.707
Smoker (n=46)	2.434±0.825	1.066±0.904
	KIT score	PDGFRA score
Female (n=2)	3±0	0.5±0.5
Male (n=52)	2.432±0.822	1.039±0.906
	KIT score	PDGFRA score
Stages I+II (n=34)	2.382±0,840	0.911±0.9193
Stages III+IV (n=20)	2.500±0.806	1.200±0.8124

There are significant differences in the both scores between smokers and non-smokers, between female and male, and between stages I+II and stages III+IV. (p>0.05) (Student's t test)

weak expression (+1) (**Figure 2C**) in 17 cases (31%), and negative expression (-) in 19 cases (35%) (**Table 1**). There was no significant difference between KIT scores and PDGFRA scores. The 5 gastric GISTs used as positive control showed strong KIT and moderate to weak PDGFRA expressions. The five uterine leiomyomas used as negative controls were negative for KIT and PDGFRA.

The survival is shown in **Table 1**. The overall median survival after initial diagnosis was 13 months (n=52). There was no significant difference in the survival between strong KIT (+3) expression cases (median survival=12 months, n=34) and cases of KIT expression less than +3 (median survival=11 months, n=18) (**Figure 3**). Likewise, there was no significant difference in the survival between PDGFRA-positive cases (median survival=11 months, n=34) and PDGFRA-negative cases (median survival=12 months, n=18) (**Figure 4**). There was a correlation between short survival and advanced stage. There were no correlations between KIT and PDGFRA expressions and smoking, gender, and disease stage (**Table 3**).

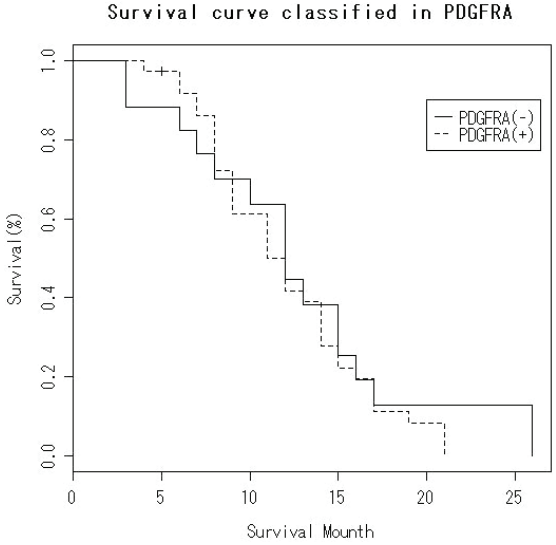


Figure 3. Kaplan-Meier survival in KIT 3+ group and KIT <3+ group. No significant difference is recognized (log-rank test).

Discussion

The sensitivity and specificity of immunohistochemistry and gene mutational status in the present study appears confirmative. The author investigated the gene status in 20 cases. Further, the results of positive and negative controls for immunohistochemistry and gene analysis confirm the immunostaining and gene analysis of the SCLC in the present study. Furthermore, the author identified many gene mutations of KIT and PDGFRA in GIST, Extra-GISTs, and germ cell tumors [20-26] which were performed in the similar or same periods. In addition, the author could not detect mutations of KIT and PDGFRA genes in extra-pulmonary small cell carcinomas [25-30].

KIT is expressed in various tumors including GIST, mast cell neoplasm, melanoma, germ cell tumor, hematopoietic malignancies, and SCLC [13, 16]. However, these reports are mainly from developed country of the white race, and such reports of the yellow race including Japan are very rare. Therefore, the author investigated *KIT* and *PDGFRA* in Japanese patients.

The KIT protein expression in SCLC varies among researchers [1-10]; it is reported to be 100% [1], 73% [2], 37% [3], 60% [4], 78% [5], 53% [6], 40% [8], 64% [9], and 30% [10]. The present case was 100%, similar to RaPoint et

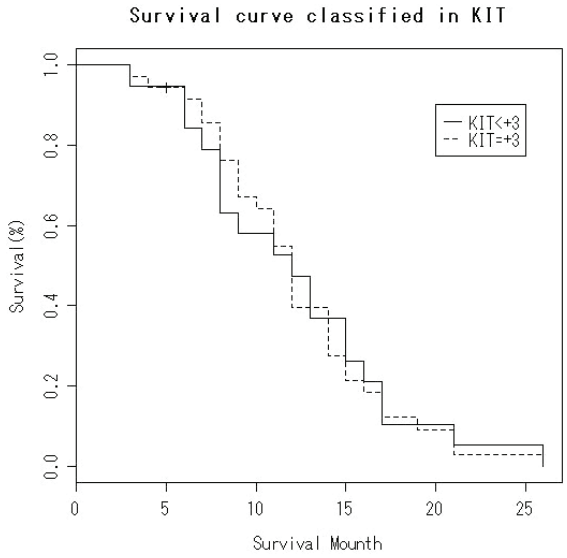


Figure 4. Kaplan-Meier survival in PDGFRA-positive group and PDGFRA-negative group. No significant difference is recognized (log-rank test).

al. [1]. The various percentages may be due to antibody used, immunohistochemical procedures, or interpretation of the immunohistochemical stains. In the present study, the author employed the sensitive Dako's Envision methods, the period of primary antibody was overnight, suggesting that the data of the present study is more accurate. In any way, the present study suggests that SCLC in Japanese patients shows high KIT protein expression. KIT expression without *KIT* gene mutations is thought to be due to *KIT* gene amplification [10].

The prognostic implications of positive KIT protein in SCLC have been controversial, and no definite conclusions have been obtained [2-6, 8]. Some authors claimed that patients with KIT-positive SCLC showed good prognosis [36], while others, in contrast, demonstrated that patients with KIT-positive SCLC showed poor prognosis [3, 6] and still others identified that there was no correlation between KIT positivity and prognosis [2]. The present study showed that there was no significant correlation in the survival between strong KIT-positive cases and weak KIT-positive cases in Japanese. More studies are required because if activating KIT mutations are present, treatment of imatinib mesylate may be effective [10, 16].

KIT mutations are frequent in GIST, acute myeloid leukemia and mast cell neoplasms [16]. With regard to SCLC, one report showed a few *KIT* mutations [8], while others indicated no *KIT* mutations in SCLC [7, 9, 10]. Boldrini et al. [8] examined exons 9 and 11 of *KIT* gene, and reported that two mutations in exon 9 and three mutations in exon 11 were found in 60 SCLC. In contrast, Sihto et al. [10] showed no mutations (exons 9, 11, 13, and 17) in 31 SCLC. Mojika et al. [7] found no *KIT* mutations (exon 17) in 23 cases of KIT-positive SCLC. Burger et al. [9] identified no *KIT* gene mutations (exon 11) in 26 SCLC. The present Japanese study showed no *KIT* mutations (exons 9, 11, 13, and 17) in 20 SCLC. Taken together, it can be concluded that *KIT* gene mutations are none or very few in SCLC.

To the best of the author's knowledge, there is only one study of *PDGFRA* mutations in SCLC [10]. Sihto et al. [10] found no *PDGFRA* mutations (exons 11 and 17) in 31 SCLC. The present case also identified no *PDGFRA* mutations (exons 12 and 18) in the 20 Japanese cases. Therefore, it can be concluded that *PDGFRA* mutations are absent in SCLC.

PDGFRA protein expression has not been reported in SCLC, to the best of the author's knowledge. The present study, for the first time, demonstrated that 35 cases of the 54 cases (65%) were positive for PDGFRA protein. This suggests that a small amount of PDGFRA protein is present in 65 % of SCLC of Japanese patients. Clinical implications of PDGFRA have been unknown. The present study, for the first time, demonstrated that there was no correlation between PDGFRA expression and survival. Much more studies are needed in PDGFRA expression and *PDFGRA* gene mutations in SCLC.

Finally, the present study showed that there were no correlations between expressions of KIT and PDGFRA and gender, smoking, and disease stage.

In summary, the present study suggests, in Japanese population, that mutations of *KIT* and *PDGFRA* genes were absent in SCLC of Japan, that KIT protein expression is present in 100%, that PDGFRA protein expression is present in 65%, and that KIT and PDGFRA protein expressions do not correlate with survival, gender, smoking, and disease stage.

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