Psf3 is a prognostic biomarker in lung adenocarcinoma

Hokka, Daisuke

Psf3 is a prognostic biomarker in lung adenocarcinoma

Running title: Psf3 expression in lung adenocarcinoma

Daisuke Hokka, MD[1]; Yoshimasa Maniwa, MD, PhD[1]; Shinya Tane, MD[1]; Wataru Nishio, MD, PhD[1]; Masahiro Yoshimura, MD, PhD[1]; Yutaka Okita, MD, PhD[2]; Chiho Ohbayashi, MD, PhD[3]; Yasuhiro Sakai, MD, PhD[3]; Xue Chen[4] and Yoshitake Hayashi, MD, PhD[4]

Divisions of [1]Thoracic Surgery and [2]Cardiovascular Surgery, Department of Surgery, Divisions of [3]Cancer Pathology and [4]Molecular Medicine and Medical Genetics, Department of Pathology, Kobe University Graduate School of Medicine, 7-5-2, Kusunoki-cho, Chuo-ku, Kobe-city, 650-0017, Japan

Corresponding author: Yoshimasa Maniwa, MD, PhD

Phone number: 8178-382-5942

Fax number: 8178-382-5959

E-mail: maniwa@med.kobe-u.ac.jp

Condensed abstract

In this study, we investigated the status of Psf3 expression in cancer cells of

125 consecutive resected lung adenocarcinomas by immunohistochemistry.

Our results indicated that increased Psf3 expression was significantly

associated with lower overall survival rate.

Abstract

Psf3 is a member of the evolutionarily conserved heterotetrameric complex

GINS (Go-Ichi-Ni-San), which consists of Sld5, Psf1, Psf2, and Psf3. Previous

studies have suggested that some GINS complex members are upregulated

in cancer, but the status of Psf3 expression in lung adenocarcinoma has not

been investigated. The objective of the current study was to determine

whether Psf3 plays a role in lung adenocarcinoma by investigating clinical

samples. We investigated the status of Psf3 expression in cancer cells of 125

consecutive resected lung adenocarcinomas by immunohistochemistry.

Increased Psf3 expression was observed in 27 (21.6%) of the 125 cases.

Further, univariate analysis and log-rank test indicated a significant association between Psf3 expression and lower overall survival rate (P = 0.0001 and P < 0.0001, respectively). Multivariate analysis also indicated a statistically significant association between increased Psf3 expression and lower overall survival rate (hazard ratio, 5.2; P = 0.0027). In a subgroup analysis of only stage I patients, increased Psf3 expression was also significantly associated with a lower overall survival rate (P = 0.0008, log-rank test). Moreover, the Ki67 index level was higher in the Psf3-positive group than in the Psf3-low positive group (P < 0.0001, Mann–Whitney U-test). Our results indicated that Psf3 can serve as a prognostic biomarker in lung adenocarcinoma.

Keywords: Psf3, GINS complex, lung adenocarcinoma, prognostic biomarker, Ki67, adjuvant therapy

1. Introduction

Psf3 is a member of the evolutionarily conserved heterotetrameric complex GINS comprising Sld5, Psf1, Psf2, and Psf3. GINS was originally identified

in *Saccharomyces cerevisiae*, and its *Xenopus laevis* homolog has been characterized in egg extracts [1-3]. In *Eukarya*, the GINS complex associates with the mini-chromosome maintenance (MCM) proteins Mcm2–7 and with Cdc45 to form the Cdc45, Mcm2-7, GINS (CMG) complex, which in turn regulates both the initiation and the progression of DNA replication [4-7]. The CMG complex constitutes the eukaryotic replicative DNA helicase and contributes to the recruitment of the replicative polymerases essential for the synthesis of leading and lagging strands [7-10]. While the GINS components that play a part in the initiation of DNA replication seem to have an important role in the accelerated DNA replication of cancer cells, the oncological significance of them is not yet clear.

Several recent reports have suggested that Psf1 is required for the acute proliferation of cells, particularly immature cells such as stem cells and progenitor cells and that this protein is useful in the successful detection of cancer stem cells [11-14]. Moreover, previous studies have suggested that some GINS complex members are upregulated in cancer, and some GINS components may be useful in the detection of cancer stem cells.

Although several studies have suggested that GINS components play a role

in cancer [15-18], the expression status of these components in lung adenocarcinoma has not yet been examined. Therefore, we sought to evaluate the expression status of Psf3 by immunohistochemical examination of surgically resected samples of human primary lung adenocarcinoma tissue. We also investigated whether Psf3 expression in tumor tissues influenced the outcome of these patients.

2. Materials and methods

2.1. Patients

The study population comprised 125 consecutive patients (71 males, 54 females) who were examined and treated at Kobe University Hospital between 2001 and 2004 for lung adenocarcinoma. All cases were underwent complete resection in this study. Of the 125 patients, 55, 27, eight, four, 23, five and three had stage IA, IB, IIA, IIB, IIIA, IIIB and IV tumors, respectively (Table1). Of the N2/N3 patients, three, one and eight patients received induction chemotherapy, radiation and chemoradiotherapy, respectively. Four patients were administered postoperative adjuvant chemotherapy. The study protocol was approved by the Regional Ethics

Committee for Clinical Research of Kobe University, and the study was conducted according to the principles of the Declaration of Helsinki. All patients provided written informed consent. Details of the clinical and demographic information, prognostic factors, and disease progression were collected retrospectively.

2.2 Immunohistochemistry

Formalin-fixed, paraffin-embedded specimens were cut at the maximal area of tumor mass into 5-µm-thick slices, and the sections were deparaffinized with xylene and rehydrated with ethanol. For antigen retrieval, the specimens were placed in Dako REALTarget Retrieval Solution (Dako, Glostrup, Denmark) at 98°C for 20 minutes. Mouse anti-human Psf3 monoclonal antibodies (1:500; GeneStem Co., Ltd., Osaka, Japan) were used as the primary antibodies for the detection of Psf3. The Dako LSAB2 Universal (DAB) kit (Dako) was used for endogenous peroxidase blocking, treatment with a secondary antibody against anti-mouse and anti-rat immunoglobulin antibody, and the visualization of HRP. Hematoxylin staining was used as the counterstain. Photographs of the stained sections

were obtained using a camera mounted on a Keyence BZ-8000 digital microscope (Keyence, Osaka, Japan).

2.3. Classification of immunohistochemical staining patterns

Immunochemically stained sections were classified by light microscopy. Because Psf3 is a nuclear protein, only sharply defined areas of HRP staining in the nuclei were judged as Psf3 staining. If HRP staining was observed in other structures, such as the cytoplasm, it was judged as background staining. Psf3 localized and functioned only in the nuclei in the previous reports and any specific staining of Psf3 in the cytoplasm was not detected in this study. This assessment method ensured objective and reproducible measurement. The ratio of the cells positive for nuclear staining in a given microscopic field (×200) was determined for each tissue sample, and the expression status was assessed on the basis of this ratio. The status of Psf3 expression as follows: if more than 50% of cancer cells in any microscopic field (×200) of tumor tissues showed nuclear staining, the tissues were considered Psf3 positive; if the ratio of positive nuclear staining was lower than 50% for all the examined microscopic fields, the tissue was

deemed Psf3 low positive.

2.4. Statistical analysis

Associations between Psf3 expression on cancer cells and clinicopathological features were determined using the χ^2-test. Survival was examined using the Kaplan–Meier method, and the significance of the difference was evaluated by a log-rank test. Variable effects on survival time were investigated using Cox's regression model. Statistical analysis was performed using the software JMP version 8 (SAS Institute, Cary, NC, USA). A threshold level of 0.05 was set for statistical evaluation.

3. Results

3.1. Psf3 expression in cancer cells of human lung adenocarcinoma

The expression status of Psf3 was determined in 125 lung adenocarcinomas and the adjacent normal lung tissues by immunohistochemistry (IHC), with the use of anti-human Psf3 monoclonal antibodies. In normal lung tissues, Psf3 expression was not detected (Fig. 1A). In some tumor tissues, the nuclei of cancer cells were stained in a scattered pattern, and the ratio of the

Psf3-positive cells was less than 10% (Fig. 1B). In contrast, some tissues

showed stained nuclei clustered in some areas of tumor tissues, and the ratio

of stained cells in such tissue samples was more than 80% (Fig. 1C). These

tissue samples showing clustered nuclear staining were classified as Psf3

positive. Thus, we determined the status of Psf3 expression as follows: if

more than 50% of cancer cells in any microscopic field (×200) of tumor tissues

showed nuclear staining, the tissues were considered Psf3 positive; if the

ratio of positive nuclear staining was lower than 50% for all the examined

microscopic fields, the tissue was deemed Psf3 low positive. Of the specimens

examined, 98 (78.4%) were low positive for Psf3, while 27 (21.6%) were

positive for Psf3 expression.

3.2. Relationship between Psf3 expression and clinicopathological

characteristics of patients

In order to evaluate the role of Psf3 in lung adenocarcinoma, we

investigated whether Psf3 expression was associated with any of

clinicopathological variables in the 125 enrolled cases of primary lung

adenocarcinoma (Table 1). The results of the analysis revealed that Psf3

expression was significantly associated with T factor (P = 0.0077), TNM stage (P = 0.0004), P factor (P = 0.0003), lymph node metastasis (P = 0.02), invasion of the pulmonary vein (P = 0.008), and cancer cell invasion of the lymphatic ducts (P = 0.02). No significant relationship was noted between Psf3 expression and age (P = 0.57), gender (P = 0.77), distant metastasis (P = 0.22), and cancer spread to the pulmonary artery (P = 0.12). These results suggest that increased Psf3 expression may enhance cancer cell proliferation and tumor progression, thereby resulting in the spread of cancer cells into the tumor vessels.

3. 3. Increased expression of Psf3 was related to poor patient prognosis

Using the data collected from 125 study patients, we evaluated their prognosis and its relationship to the expression of Psf3. Follow-up data of all the 125 cases were available, for at least 5 years after surgery. We examined the overall survival (OS) of Psf3-low positive and Psf3-positive groups and found a statistically significant difference between the 2 groups by using the log-rank test (P < 0.0001). The survival of Psf3-low positive patients was greater than that of the Psf3-positive patients (Fig. 2A). A univariate

analysis indicated that among the clinicopathological factors, gender (male),

tumor classification, lymph node metastasis, invasion of the pulmonary vein,

and increased Psf3 expression correlated with the outcome (Table 2). Further

assessment using the Cox multivariate analysis indicated that gender (male),

lymph node metastasis, and increased Psf3 expression were statistically

significant predictors for poor OS (Table 2).

3. 4. Increased expression of Psf3 was also related to poor patient prognosis

in stage I lung adenocarcinoma

In the current study, we analyzed the association of clustered Psf3

expression in stage I lung adenocarcinoma. Among the stage I cases, 9

(11.0%) and 73 (89.0%) patients were classified as Psf3 positive and Psf3 low

positive, respectively (Table 1). A survival analysis that included only stage I

patients revealed that the OS curve for the Psf3-positive group was lower

than that for the Psf3-low positive group. The log-rank test showed that the

intergroup difference was statistically significant ($P = 0.0008$; Fig. 2B).

3. 5. Relationship between Psf3 expression and Ki67 index

We examined the relationship between increased Psf3 expression and cancer cell proliferation. We used the Ki67 (MIB-1) expression index as an indicator of cell proliferation. In this study, the Ki67 index was calculated using the maximal section of the tumor mass. Using the Mann–Whitney U-test, the Ki67 index level was found to be higher in the Psf3-positive group than in the Psf3-low positive group. The median Ki67 index was 5% and 17% in the Psf3-low positive and Psf3-positive tumors, respectively (Fig. 3).

Additionally, immunochemical staining of Ki67 was also performed on serial sections that were used for Psf3 staining. The ratio of Ki67-positive cancer cells was found to be higher in areas where excessive staining of the nuclei of cancer cells was observed when tested with the Psf3 antibody (Fig. 1C, D). However, different staining patterns were observed for Ki67 and Psf3. 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 less than 50% (Fig. 1D).

3. 6. Psf3: most powerful predictor of poor prognosis in lung adenocarcinoma patients

We have previously performed IHC for 10 cancer-related proteins (CDC45, HIF1, sirt1, E-cadhelin, Nectin3, and the proteins listed in Table 3 other than Psf3) with the same paraffin-embedded specimens of the 125 cases investigated in this study [19, 20] and examined the relationship between their expression in cancer cells and the prognosis of the patients. Univariate analysis revealed the significant association of 5 of the proteins with poor prognosis. To clarify the prognostic value of Psf3 expression in cancer cells, we statistically compared the expression levels of these 5 proteins and Psf3. Multivariate analysis revealed that Psf3 was the strongest predictor of poor prognosis (Table 3).

4. Discussion

Psf3 is a member of the GINS complex, along with Sld5, Psf1, and Psf2. Psf1 is tightly regulated at the transcriptional level in stem cells and enables the successful detection of cancer stem cells [11-14]. Therefore, it seems reasonable that other GINS components may also facilitate the detection of cancer stem cells in tumors. Cancer stem cells, which are resistant to anti-cancer drugs and irradiation, appear to be responsible for tumor growth

in hematological and solid cancers. The detection of these cells is critical for identifying molecular targets to inhibit their growth. We conducted 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).

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, 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) were 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 statement

Acknowledgments

References

[1] Takayama Y, Kamimura Y, Okawa M, Muramatsu S, Sugino A, Araki H. GINS, a novel multiprotein complex required for chromosomal DNA replication in budding yeast. Genes Dev. 2003;17:1153–65.

[2] Kanemaki M, Sanchez-Diaz A, Gambus A, Labib K. Functional proteomic identification of DNA replication proteins by induced proteolysis in vivo. Nature 2003;423:720–24.

[3] Kubota Y, Takase Y, Komori Y, Hashimoto Y, Arata T, Kamimura Y, et al.

A novel ring-like complex of Xenopus proteins essential for the initiation of DNA replication. Genes Dev. 2003;17:1141–52.

[4] Moyer SE, Lewis PW, Botchan M. Isolation of the Cdc45/Mcm2–7/GINS (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase. Proc Natl Acad Sci USA. 2006;103:10236–41.

[5] Gambus A, Jones RC, Sanchez-Diaz A, Kanemaki M, van Deursen F, Edmondson RD, et al. GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks. Nat Cell Biol. 2006;8:358–66.

[6] Pacek M, Tutter AV, Kubota Y, Takisawa H, Walter JC. Localization of MCM2–7, Cdc45, and GINS to the site of DNA unwinding during eukaryotic DNA replication. Mol Cell. 2006;21:581–7.

[7] Bermudez V, Farina A, Raghavan V, Tappin I, Hurwitz J. Studies on human DNA polymerase epsilon and GINS complex and their role in DNA replication. J Biol Chem. 2011;286:28963–77.

[8] Kang YH, Galal WC, Farina A, Tappin I, Hurwitz J. Properties of the human Cdc45/Mcm2-7/GINS helicase complex and its action with DNA polymerase epsilon in rolling circle DNA synthesis. Proc Natl Acad Sci

USA. 2012;109:6042–7.

[9]   Kamada K, Kubota Y, Arata T, Shindo Y, Hanaoka F. Structure of the human GINS complex and its assembly and functional interface in replication initiation. Nat Struct Mol Biol. 2007;14:388–96.

[10] De Falco M, Ferrari E, De Felice M, Rossi M, Hubscher U, Pisani FM. The human GINS complex binds to and specifically stimulates human DNA polymerase alpha-primase. EMBO Rep. 2007;8:99–103.

[11] Ueno M, Itoh M, Kong L, Sugihara K, Asano M, Takakura N. Psf1 is essential for early embryogenesis in mice. Mol Cell Biol. 2005;25:10528–32.

[12] Ueno M, Itoh M, Sugihara K, Asano M, Takakura N. Both alleles of Psf1 are required for maintenance of pool size of immature hematopoietic cells and acute bone marrow regeneration. Blood 2009;113:555–62.

[13] Han Y, Ueno M, Nagahama Y, Takakura N. Identification and characterization of stem cell-specific transcription of Psf1 in spermatogenesis. Biochem Biophys Res Commun. 2009;280:609–13.

[14] Nagahama Y, Ueno M, Miyamoto S, Morii E, Minami T, Mochizuki N, et al. Psf1, a DNA replication factor expressed widely in stem and

progenitor cells, drives tumorigenic and metastatic properties. Cancer Res. 2010;70:1215–24.

[15] Obama K, Ura K, Satoh S, Nakamura Y, Furukawa Y. Up-regulation of Psf2, a member of the GINS multiprotein complex, in intrahepatic cholangiocarcinoma. Oncol Rep. 2005;14:701–6.

[16] Ryu B, Kim DS, Deluca AM, Alani RM. Comprehensive expression profiling of tumor cell lines identifies molecular signatures of melanoma progression. PLoS One. 2007;2:e594.

[17] Nagahama Y, Ueno M, Haraguchi N, Mori M, Takakura N. Psf3 marks malignant colon cancer and has a role in cancer cell proliferation. Biochem Biophys Res Commun. 2010;5:150–4.

[18] Nakahara I, Miyamoto M, Shibata T, Akashi-Tanaka S, Kinoshita T, Mogushi K, et al. Up-regulation of Psf1 promotes the growth of breast cancer cells. Genes Cells. 2010;15:1015–24.

[19] Satoh N, Maniwa Y, Bermudez VP, Nishimura K, Nishio W, Yoshimura M, et al. Oncogenic phosphatase Wip1 is a novel prognostic marker for lung adenocarcinoma patient survival. Cancer Sci. 2011;102:1101–6.

[20] Nakai R, Maniwa Y, Tanaka Y, Nishio W, Yoshimura M, Okita Y, et al.

Overexpression of Necl-5 correlates with unfavorable prognosis in patients with lung adenocarcinoma. Cancer Sci. 2010;101:1326–30.

[21] Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. J Cell Physiol. 2000;182:311–22.

[22] Landberg G, Tan EM, Roos G. Flow cytometric multiparameter analysis of proliferating cell nuclear antigen/cyclin and Ki-67 antigen: a new view of the cell cycle. Exp Cell Res. 1990;187:111–8.

Table 1. Association between increased expression of Psf3 and clinicopathological characteristics in 125 patients with lung adenocarcinoma

Variable	Total	Psf3		P-value
		low positive	positive	
No. patients (%)	125	98	27	NA
Age in years, mean ± SD (range)	67.4 ± 8.8 (42–84)	67.6 ± 8.6 (42–84)	66.5 ± 9.6 (42–81)	0.573
Gender Male/female	71/54	55/43	16/11	0.770
T factor T1/T2/T3/T4	69/42/4/10	60/29/1/8	9/13/3/2	0.0077*
N factor N0/N1/N2/N3	87/13/24/1	76/8/14/0	11/5/10/1	0.0013*
M factor M0/M1	122/3	97/1	25/2	0.226
Stage I/II/III/IV	82/12/28/3	73/7/17/1	9/5/11/2	0.0004*
P factor 0/1/2/3	86/19/12/8	72/17/7/2	14/2/5/6	0.0003*
PA invasion Negative/positive	101/24	82/16	19/8	0.120
PV invasion Negative/ positive	74/51	64/34	10/17	0.008*
LY invasion Negative/ positive	75/50	64/34	11/16	0.021*

Abbreviations: LY, lymphatic duct; NA, not applicable; PA, pulmonary artery; PV, pulmonary vein

*Significant P-value

Table 2. Univariate and multivariate analyses of the association between the overall survival of 125 patients with lung adenocarcinoma and prognostic factors by Cox proportional hazard models

Variable	Hazard ratio	95% Confidence interval	P-value
Univariate			
Age	1.01	0.96–1.06	0.621
Gender (male versus female)	3.21	1.26–8.20	0.014*
T factor (T1<)	3.27	1.38–7.78	0.0071*
LN (negative versus positive)	5.62	2.32–13.5	0.0001*
PV invasion (negative versus positive)	3.40	1.44–8.00	0.0051*
Psf3 (low positive versus positive)	6.91	2.70–17.6	0.0001*
Multivariate			
Age	1.03	0.97–1.09	0.260
Gender(male versus female)	4.27	1.36–13.4	0.012*
T factor (T1<)	2.30	0.78–6.75	0.129
LN (negative versus positive)	4.08	1.32–12.5	0.014*
PV invasion (negative versus positive)	0.92	0.29–2.88	0.886
Psf3 (low positive versus positive)	5.22	1.77–15.3	0.0027*

Abbreviations: LN, lymph node metastasis; PV, invasion of the pulmonary vein

*Significant P-value

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*

Abbreviations: γ-H2AX, phosphorylated H2AX; p53, tumor protein p53; Necl-5, Nectin-like molecule-5; Wip1, Wild-type p53-induced phosphatase 1

*Significant P value

Fig 1A. Psf3-negative normal tissues
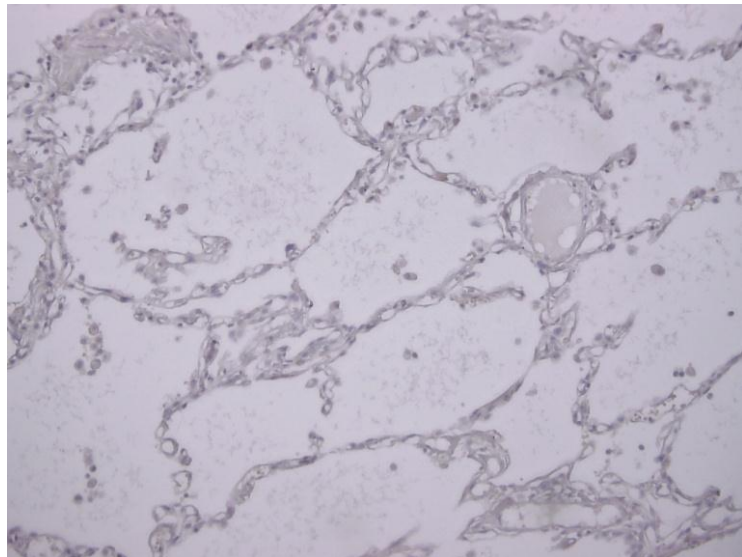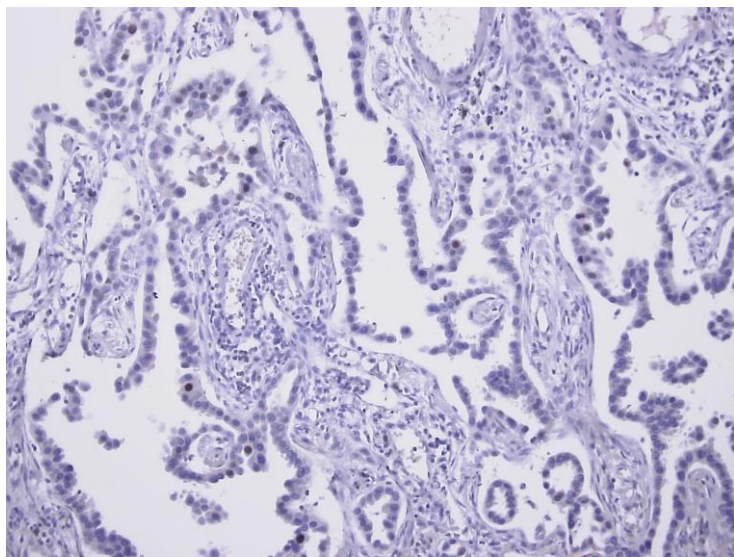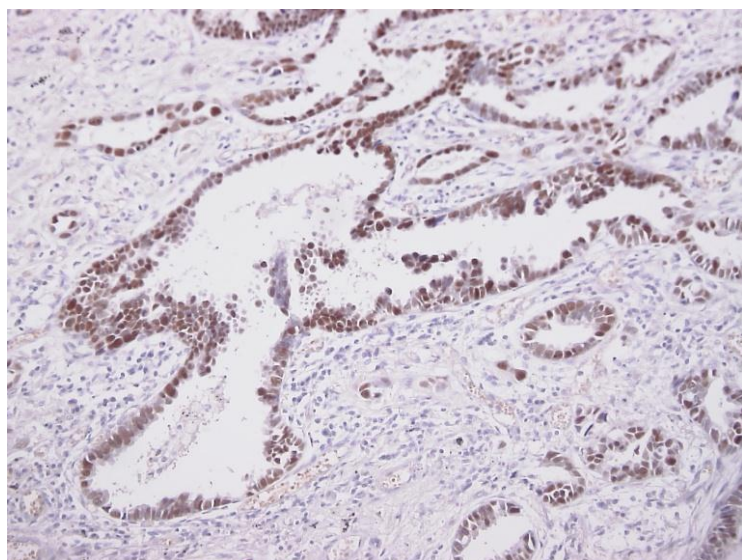
Fig 1B. Psf3-low positive tumor tissues

Fig 1C. Psf3-positive tumor tissues
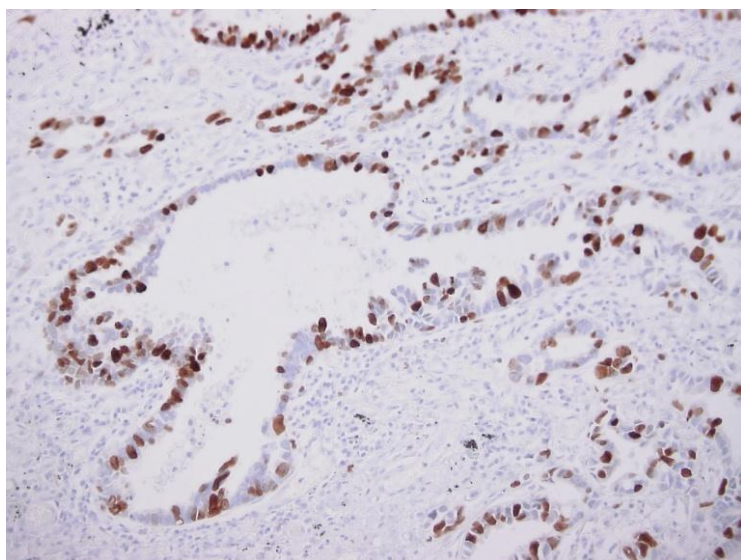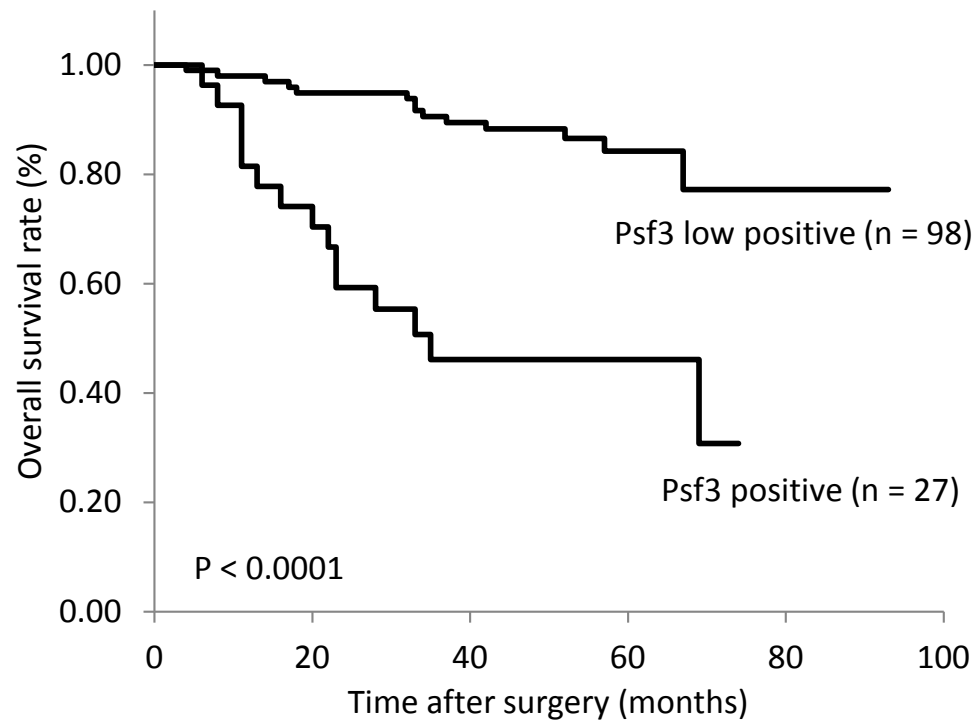
Fig 1D. Ki67
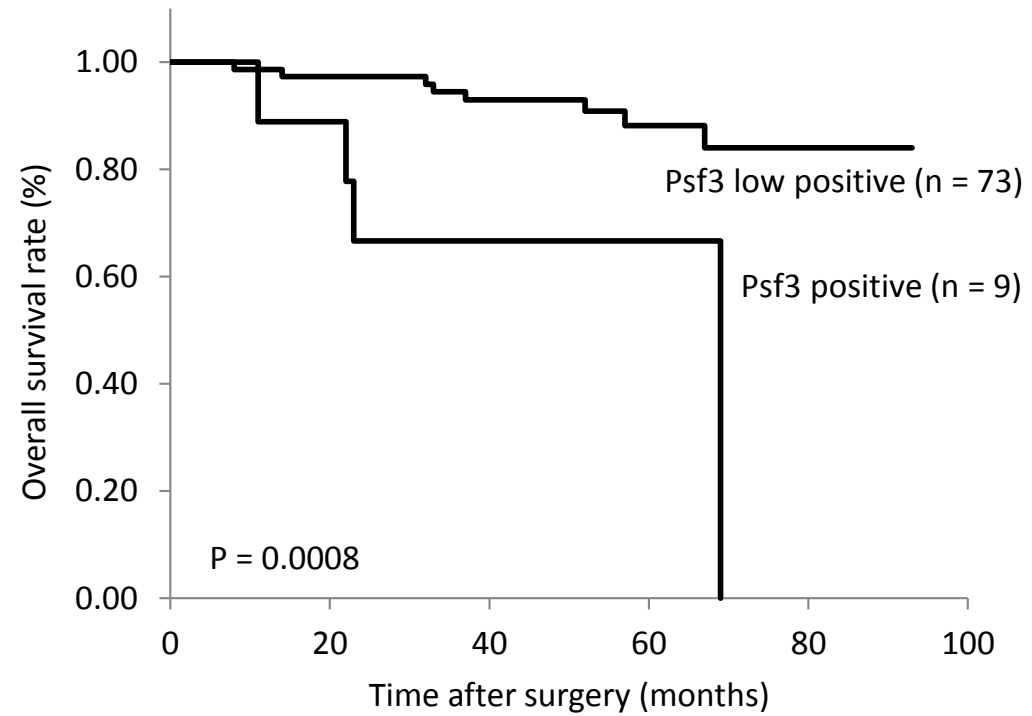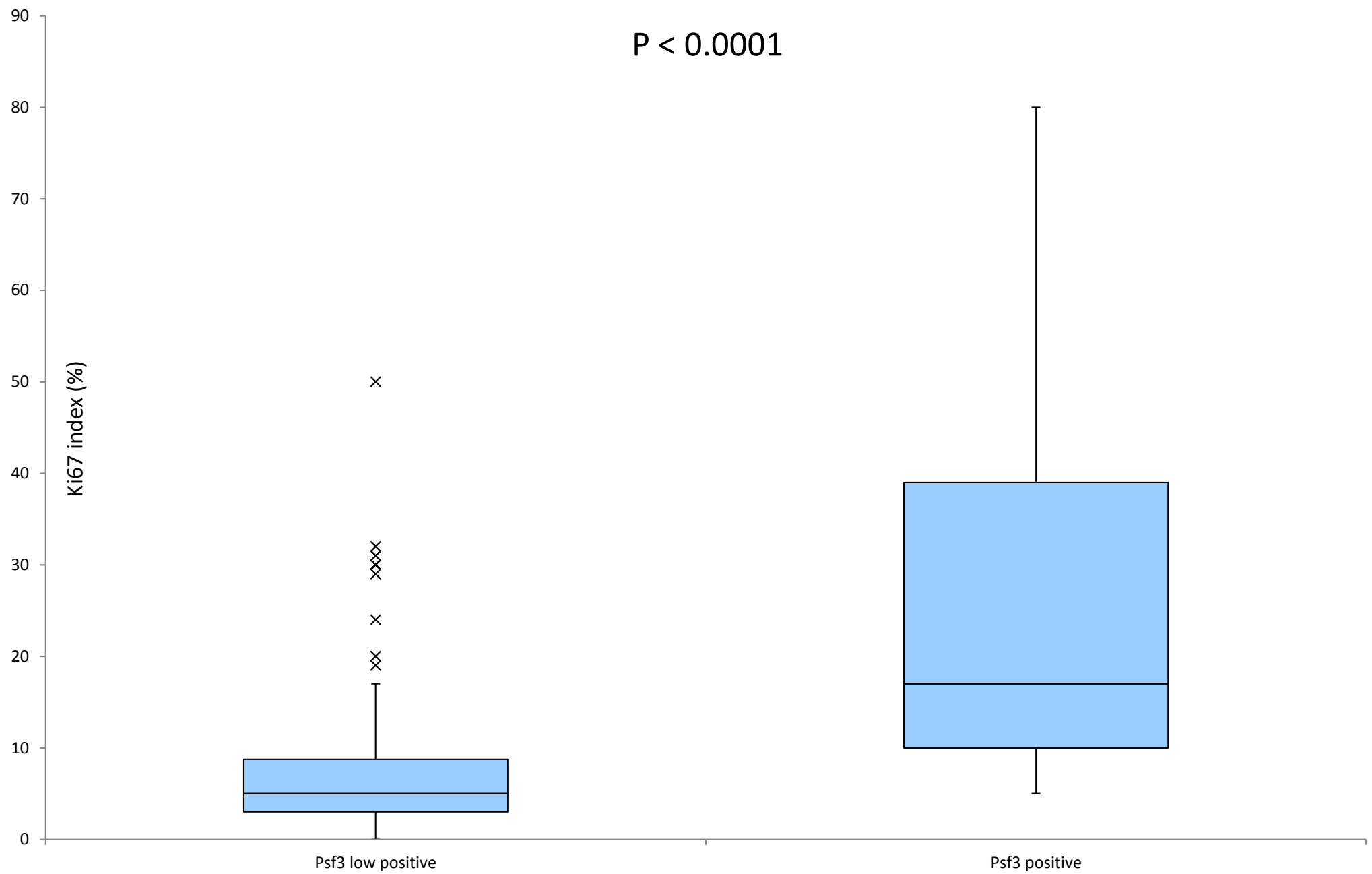
Figure 2.

Fig 2A.

Fig 2B.

Figure 3.

Figure legends

Figure 1. An immunohistochemical analysis of the Psf3 expression status in cancer cells of human primary lung adenocarcinoma is illustrated. (A) Psf3-negative normal lung tissues. The staining of nuclei was not detected in epithelial or interstitial tissue. (B) Psf3-low positive tumor tissues. The nuclei of cancer cells were stained in a scattered pattern. The ratio of the positive cells was less than 10% (C) Psf3-positive tumor tissues. The stained nuclei were clustered at some regions of the tumor tissues. The ratio of the stained cells in these areas was more than 80%. (D) Immunohistochemical analysis of the expressions of Psf3 and Ki67 in serial sections. While almost all nuclei of the cancer cells were stained with the Psf3 antibody, Ki67 staining was observed in a scattered pattern in the same areas. Ki67 index; 40%.

Figure 2. (A) Kaplan–Meier plot of the overall survival rate in 125 patients with lung adenocarcinoma and its relationship to the Psf3 expression status. *P*-value was determined using the log-rank test. (B) Kaplan–Meier plot of the overall survival rate in 82 patients with lung adenocarcinoma and its

relationship to the Psf3 expression status in stage I patients. *P*-value was

determined using the log-rank test.

Figure 3. Ki67 index (%) in lung adenocarcinoma samples and its

relationship to the Psf3 expression status. *P*-value was determined using the

Mann–Whitney *U*-test.

Conflict of Interest statement

None declared