The immunoreaction was amplified with a Vectastain ABC kit (Vector Laboratories) and visualized by incubation with a 3,3′-diaminobenzidine solution acting as a chromogen. The sections were then counterstained with hematoxylin and dehydrated. Images were captured using a microscope at a magnification of ×200 (model BZ-9000; Keyence) and the area of CD31-positive vessel-like structures was measured in five random microscopic fields per section using Dynamic cell count software BZ-HIC (Keyence).

Proliferation assay

HUVECs were suspended in medium (1×10^4/100 μL) containing 10 ng/mL VEGF plus SK-216 at various concentrations. The cells were seeded into a 96-well tray and incubated. To determine cells' proliferation rates after 16 and 36 hours, the absorbance of the medium in each well was assessed using cell counting kit-8 (DOJINDO) following the manufacturer's instructions.

Cell migration assay

HUVEC migration was assessed using an Oris Universal Cell Migration Assembly kit (Platypus Technologies) following the manufacturer's instructions. Briefly, HUVECs (2×10^4) suspended in 100 μL of medium were seeded into each test well of the Oris plate with the well inserts (stoppers) and then incubated to allow cell attachment. After 4 hours, the stoppers in each well were removed. HUVECs were incubated with 10 ng/mL VEGF and SK-216 at various concentrations for 36 hours, and then were stained with calcein acetoxymethylester (Calcein AM) stock solution (2 mmol/L; DOJINDO). Images were captured at a magnification of ×40 using a fluorescence microscope (model BZ-9000; Keyence) and the areas occupied by HUVECs (occupied area) and not occupied by HUVECs (background area) were measured using Dynamic cell count software BZ-HIC (Keyence). The percentage of occupied area was determined by the following formula: 100 × (background area at baseline − background area at 36 hours)/background area at baseline.

Capillary-like tube formation assay

Seventy microliters of Matrigel was applied to each well of a 96-well plate and incubated for 30 minutes. HUVECs (1×10^4) suspended in 100 μL of medium were plated onto the Matrigel and incubated with 10 ng/mL of VEGF and SK-216 at various concentrations for 16 hours and then were stained with Calcein AM stock solution (2 mmol/L). Images were captured at a magnification of ×20 using a fluorescence microscope (model BZ-9000) and the total area of the tube-like space was quantified using Dynamic cell count software BZ-HIC (Keyence).

Statistical analysis

Statistical analyses were undertaken using SPSS 17 (SPSS Japan). All the results are expressed as mean ± SEM, and the Student *t* test or Mann–Whitney *U* test were used to evaluate statistical differences between the groups. A *P* value more than 0.05 was considered to be statistically significant.

Results

Oral administration of SK-216, a PAI-1–specific inhibitor, reduced tumor progression in both the subcutaneous tumor model and the tail vein metastasis model

To determine whether PAI-1 could be a therapeutic target in the treatment of malignancy, a subcutaneous tumor model and a tail vein metastasis model were generated in C57BL/6 mice using C57BL/6–derived cell lines, LLC, and B16 melanoma cells. SK-216 was orally administered to the mice. Interestingly, B16 cells were found to secrete almost no PAI-1 in contrast with LLC cells (Fig. 1A). In consistence with the PAI-1 secretion levels *in vitro*, immunohistochemical staining of PAI-1 for subcutaneous tumors in PAI-$1^{-/-}$ mice confirmed that PAI-1 was detectable in the tumor of LLC cells but not in that of B16 cells (Supplementary Fig. S2). The volumes of subcutaneous tumors were evaluated 14 days after the inoculation of LLC or B16 cells and the number of tumor nodules on lung surfaces was counted 21 days after injection. As shown in Fig. 1B and D, the volumes of subcutaneous tumors 14 days after the inoculation of LLC and B16 cells were significantly smaller in the SK-216–treated group than in the control group. In addition, the number of lung tumor nodules 21 days after the injection of LLC or B16 cells was significantly lower in the SK-216–treated group than in the control group (Fig. 1C and E). Interestingly, the effects of SK-216 on subcutaneous tumor growth in the subcutaneous tumor model showed a trend toward dose-dependency (Fig. 1B).

SK-216 reduced the degree of angiogenesis in subcutaneous tumor

To determine whether SK-216 affected the degree of angiogenesis in subcutaneous tumor, we undertook immunohistochemical staining of the excised subcutaneous tumors of LLC and B16 cells with anti-CD31 monoclonal antibody (mAb). In both subcutaneous tumors of LLC and B16 cells in WT mice, the areas of CD31-positive vessels were significantly lower in the SK-216–treated group than in the control group (Fig. 2A and C).

Host but not tumor PAI-1 was crucial for tumor progression in the subcutaneous tumor model and the tail vein metastasis model

The systemic administration of SK-216 effectively reduced the size of subcutaneous tumors and the extent of lung metastases regardless of the presence or absence of PAI-1 secretion by the tumor cells. To further investigate the significance of tumor PAI-1 in tumor progression, we established two LLC-derived cell lines that differed in expression levels of PAI-1, namely siControl LLC and siPAI-1 LLC cells. As shown in Fig. 3A, qRT-PCR revealed that the expression level of PAI-1 mRNA was significantly decreased in siPAI-1 LLC cells compared with siControl

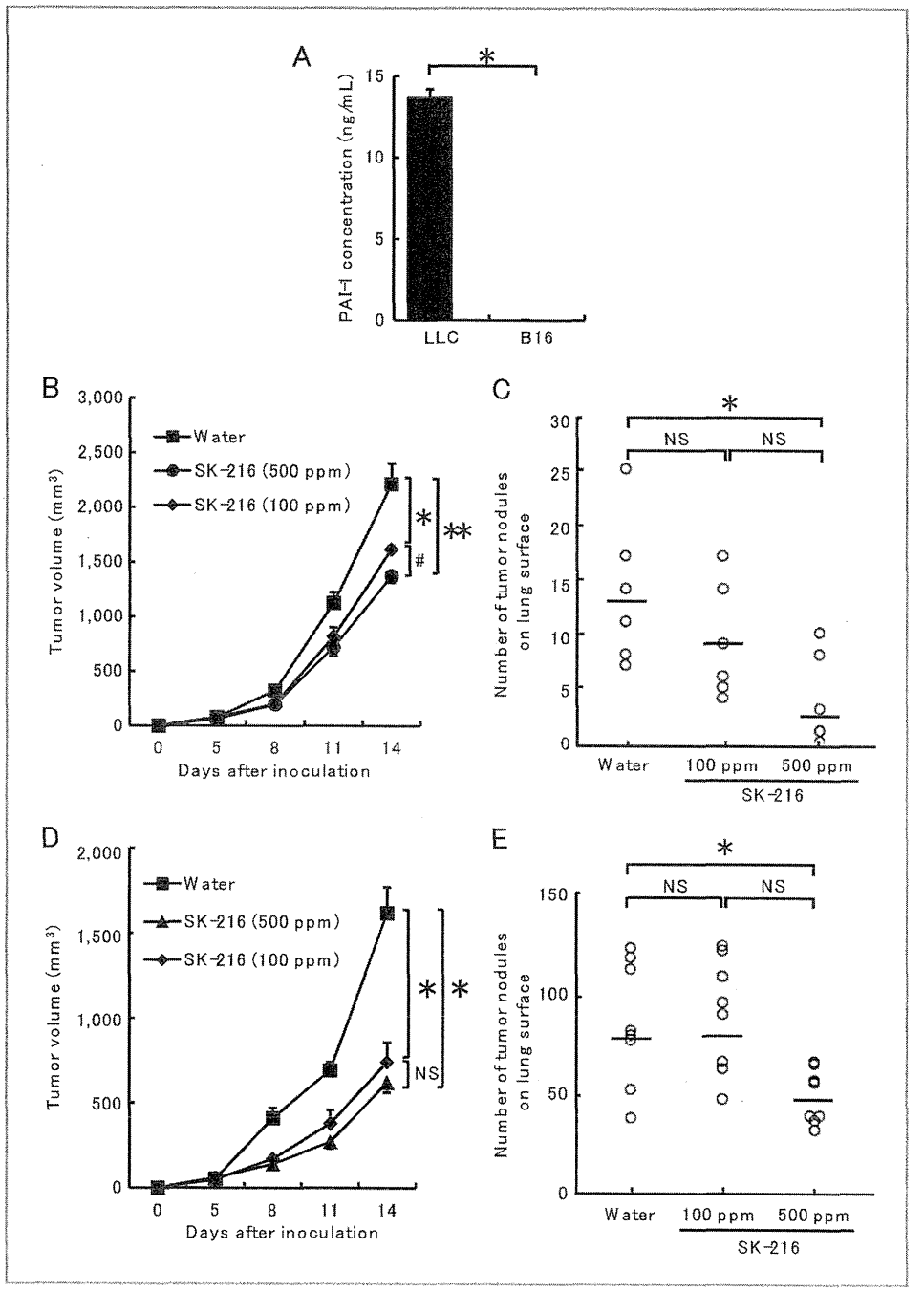

Figure 1. Effects of systemic administration of SK-216 on tumor progression in the subcutaneous tumor model and the tail vein metastasis model. A, comparison of PAI-1 secretion levels between LLC and B16 cells. LLC or B16 cells (1×10^4) were seeded in 96-well plates and cultured for 24 hours. Concentrations of PAI-1 in culture media were measured by ELISA. Data represent the mean values (±SEM) of triplicate samples and were analyzed with the Student t test. *, $P < 0.01$ versus LLC cells. B and D, evaluation of tumor sizes in the subcutaneous tumor model using PAI-1-secreting LLC cells and PAI-1-nonsecreting B16 cells. Volumes of subcutaneous tumors were measured twice a week for 2 weeks after the inoculation of LLC (B) or B16 (D) cells into WT mice. Mice were given drinking water or SK-216 (100 or 500 ppm). The data represent the mean values (±SEM) of 6 mice per group and were analyzed with the Student t test. *, $P < 0.01$; **, $P < 0.05$ versus control group. #, $P < 0.05$ versus the group treated with 100 ppm of SK-216. NS, not significant. C and E, evaluation of lung metastases in the tail vein metastasis model using PAI-1-secreting LLC cells or PAI-1-nonsecreting B16 cells. The number of tumor nodules on the lung surface of WT mice was counted 21 days after injection of LLC (C) or B16 (E) cells through the tail vein. Mice were given drinking water or SK-216 (100 or 500 ppm). Each bar represents the mean value of 6 or 8 mice per group. The data were analyzed with the Student t test. *, $P < 0.05$ versus control group. NS, not significant.

LLC cells. Similarly, PAI-1 protein levels in the culture media were approximately one third decreased in siPAI-1 LLC cells compared with siControl LLC cells (Fig. 3B). *In vitro*, no differences in proliferation or migration between siControl LLC and siPAI-1 LLC cells were shown (data not shown).

Next, to determine the relationship between host and tumor PAI-1 in tumor growth, siControl LLC or siPAI-1 LLC cells were subcutaneously inoculated or injected through the tail vein into WT or PAI-$1^{-/-}$ mice. As shown in Fig. 3C, the volumes of subcutaneous tumors 14 days after the inoculation of siControl LLC or siPAI-1 LLC cells were significantly smaller in PAI-$1^{-/-}$ mice than in WT mice. In WT mice, there were no significant differences in the volumes of subcutaneous tumors when inoculated with siControl LLC or siPAI-1 LLC cells. The same outcomes were observed in PAI-$1^{-/-}$ mice. Downregulated expression of PAI-1 was confirmed in subcutaneous tumors initiated by siPAI-1 LLC cells compared with that of siControl LLC cells as determined by immunohistochemistry (Supplementary Fig. S3). Similar to the results in the subcutaneous tumor model, in the tail vein metastasis

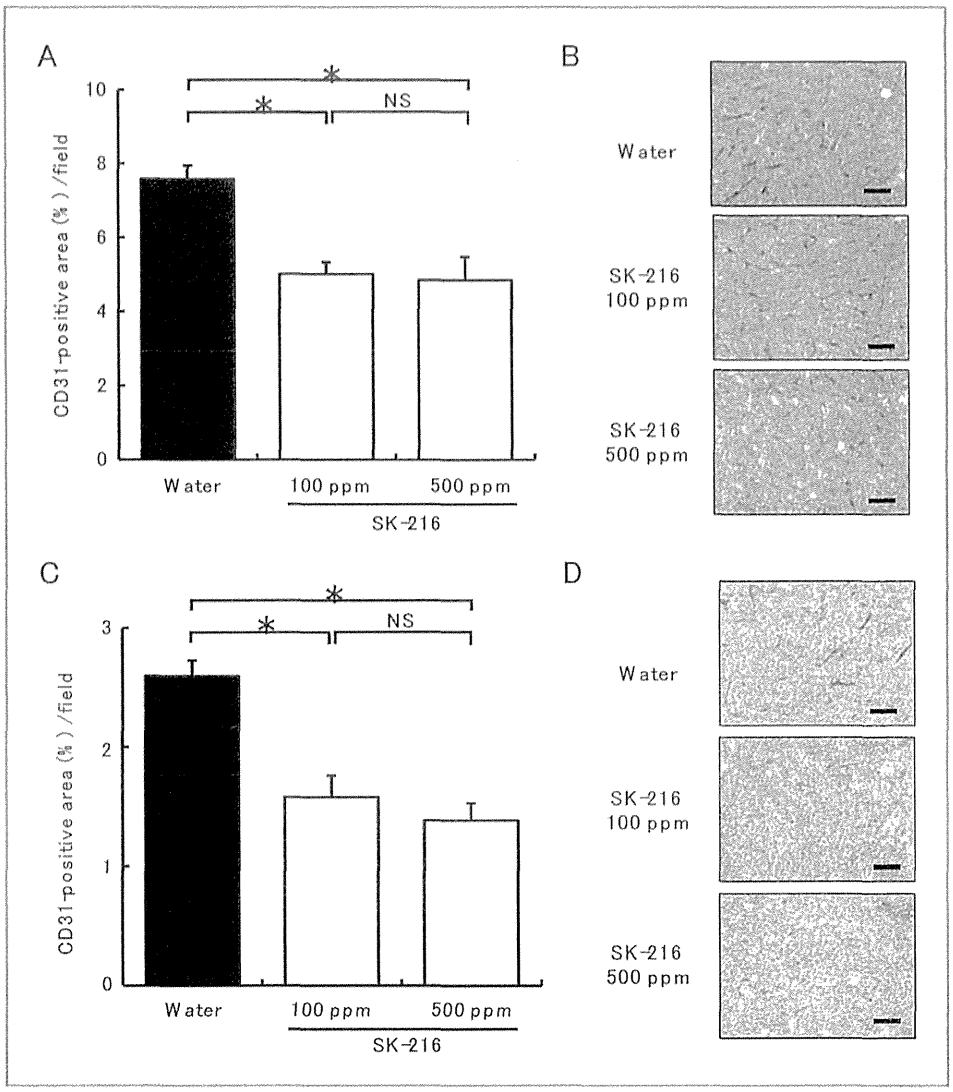

Figure 2. Evaluation of angiogenesis in subcutaneous tumors of WT mice subcutaneously inoculated with LLC (A) or B16 (C) cells. Mice were given either water or SK-216 (100 or 500 ppm). The area of CD31-positive vessels was calculated as described in Materials and Methods. Data represent the mean values (±SEM) of 6 mice in each group and were analyzed with the Student t test. *, $P < 0.01$ versus control group. NS, not significant. B and D, representative immunohistochemical staining of CD31 in subcutaneous tumors. Scale bar, 100 μm.

model, the number of tumor nodules on the lung surface 21 days after the injection of cells was significantly lower in PAI-$1^{-/-}$ mice than in WT mice (Fig. 3D). In both WT and PAI-$1^{-/-}$ mice, there were no significant differences in the numbers of lung nodules between siControl LLC and siPAI-1 LLC cells. These results strongly suggest that host PAI-1 but not tumor PAI-1 is the determinant for the degree of tumor progression in both the subcutaneous tumor model and the tail vein metastasis model. To substantiate the significance of host PAI-1 in tumor progression, similar models were generated in WT and PAI-$1^{-/-}$ mice using PAI-1–nonsecreting B16 cells. As shown in Fig. 3E and F, the volumes of subcutaneous tumors and the number of lung surface nodules were significantly smaller in PAI-$1^{-/-}$ mice than in WT mice.

Deficiency of host PAI-1 reduced the degree of tumor angiogenesis

Previous studies clearly showed the important role of host PAI-1 in tumor angiogenesis (11–14). To confirm the significance of host PAI-1 in tumor angiogenesis, the extent of angiogenesis in subcutaneous tumors of PAI-1–secreting LLC cells and nonsecreting B16 cells were compared between WT and PAI-$1^{-/-}$ mice. Immunohistochemical staining of the excised subcutaneous tumor sections with anti-CD31 mAb showed apparently reduced areas of CD31-positive vessels in both subcutaneous tumors of LLC cells and B16 cells in PAI-$1^{-/-}$ mice compared with those in WT mice (Fig. 4A and C).

Host but not tumor PAI-1 was determinant for the effects of SK-216 on tumor growth and angiogenesis

To evaluate by which of host or tumor PAI-1 the antitumor effect of SK-216 was more affected, WT and PAI-$1^{-/-}$ mice subcutaneously inoculated with siControl LLC or siPAI-1 LLC cells were treated or untreated with SK-216. As shown in Fig. 5A, the volumes of subcutaneous tumors in WT mice were significantly smaller in the SK-216–treated groups than in the untreated groups. However, these differences were not observed in PAI-$1^{-/-}$

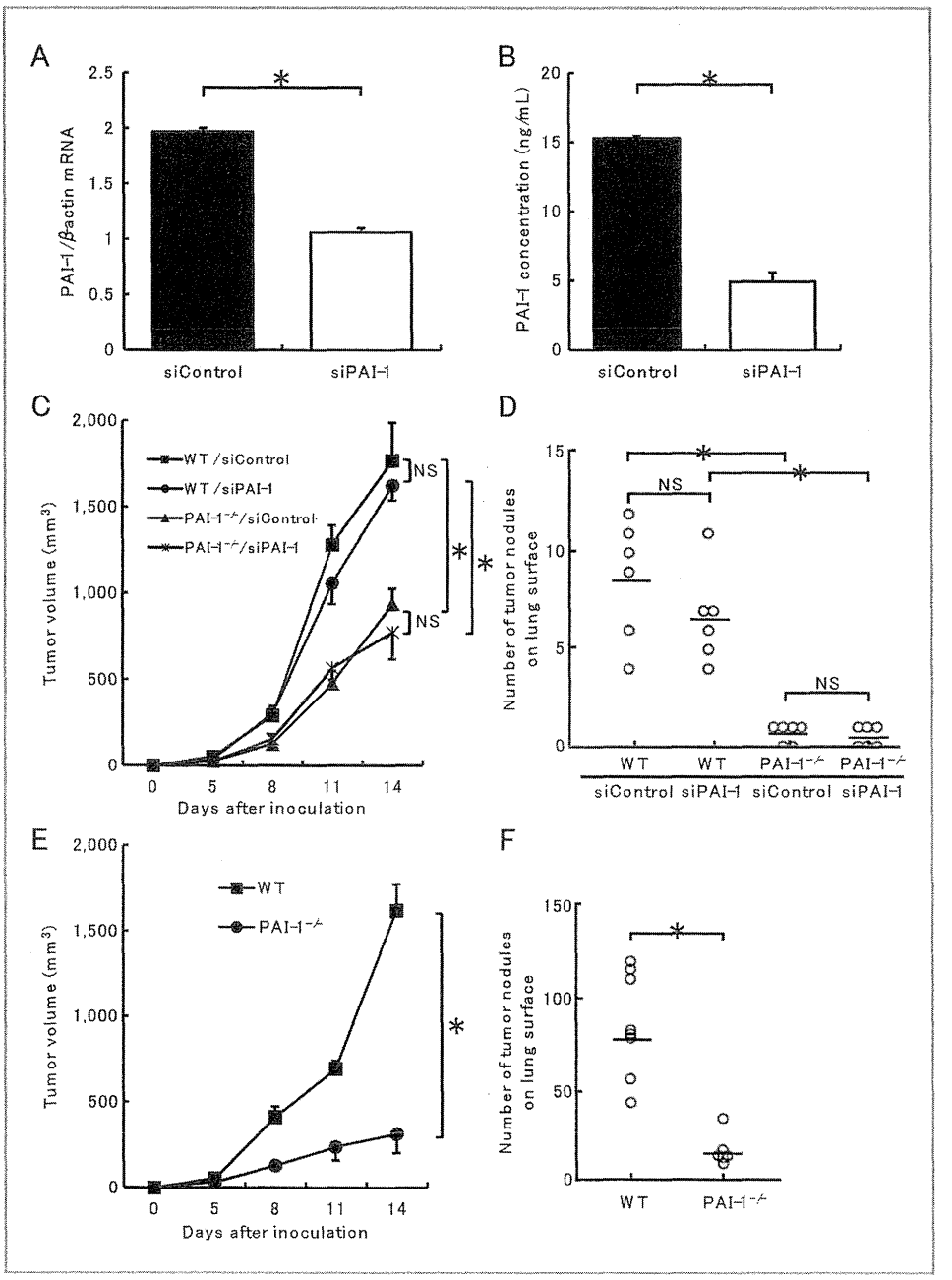

Figure 3. Evaluation of the knockdown efficiency of PAI-1 in LLC cells stably transfected with siRNA against PAI-1. A, siControl and siPAI-1 LLC cells were established as described in Materials and Methods. Expression levels of PAI-1 mRNA in siControl and siPAI-1 LLC cells were evaluated by qRT-PCR. B, concentrations of PAI-1 in culture media of siControl and siPAI-1 LLC cells were measured by ELISA. Data represent the mean values (±SEM) of triplicate samples and were analyzed by the Student t test. *, $P < 0.01$ versus siControl LLC cells. Effects of host and/or tumor PAI-1 expression levels on tumor progression in two tumor models. C, evaluation of tumor sizes in a subcutaneous tumor model using siControl and siPAI-1 LLC cells. Volumes of subcutaneous tumors were measured twice a week for 2 weeks after inoculation of siControl or siPAI-1 LLC cells into WT or PAI-$1^{-/-}$ mice. Data represent the mean values (±SEM) of 6 mice per group and were analyzed with the Mann-Whitney U test. *, $P < 0.01$ versus WT mice. NS, not significant. D, evaluation of lung metastases in the tail vein metastasis model using siControl and siPAI-1 LLC cells. The number of tumor nodules on the lung surface of WT and PAI-$1^{-/-}$ mice was counted 21 days after injection of siControl or siPAI-1 LLC cells through the tail vein. Each bar represents the mean value of 6 mice per group. The data were analyzed with the Mann-Whitney U test. *, $P < 0.01$ versus WT mice. NS, not significant. E, evaluation of tumor sizes in the subcutaneous tumor model using B16 cells. Volumes of subcutaneous tumors were measured twice a week for 2 weeks after the inoculation of B16 cells into WT or PAI-$1^{-/-}$ mice. Data represent the mean values (±SEM) of 6 mice per group and were analyzed with the Mann-Whitney U test. *, $P < 0.01$ versus WT mice. F, evaluation of lung metastases in the tail vein metastasis model using B16 cells. The number of tumor nodules on the lung surface of WT and PAI-$1^{-/-}$ mice was counted 21 days after injection of B16 melanoma cells through the tail vein. Each bar represents the mean value of 6 or 8 mice per group. The data were analyzed with the Mann-Whitney U test. *, $P < 0.01$ versus WT mice.

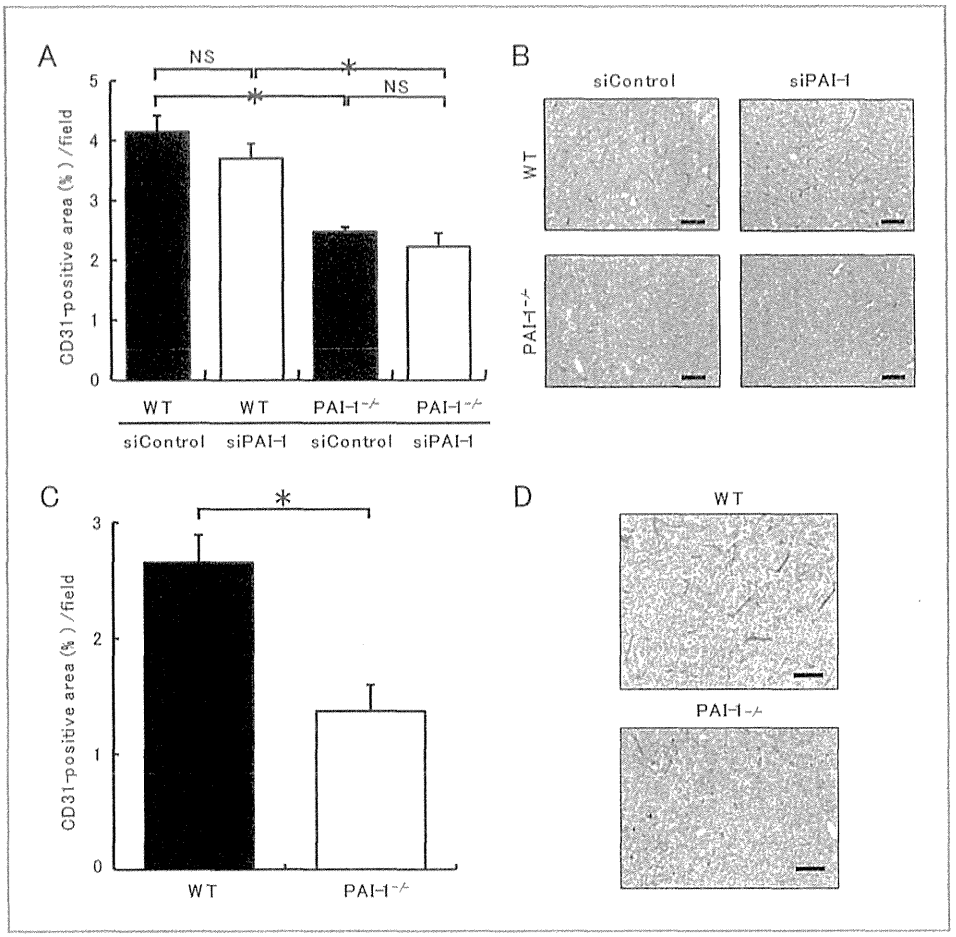

Figure 4. Evaluation of angiogenesis in subcutaneous tumors of WT and PAI-$1^{-/-}$ mice subcutaneously inoculated with siControl or siPAI-1 LLC cells (A) or B16 cells (C). The area of CD31-positive vessels was calculated as described in Materials and Methods. Data represent the mean values (±SEM) of 6 mice per group and were analyzed with the Student t test. *, $P < 0.01$ versus WT mice. NS, not significant. B and D, representative immunohistochemical staining for CD31 in a subcutaneous tumor. Scale bar, 100 μm.

mice (Fig. 5B). In both WT and PAI-$1^{-/-}$ mice, there were no significant differences in the volumes of subcutaneous tumors between siControl LLC and siPAI-1 LLC cells (Fig. 5A and B). Similar to the results of subcutaneous tumor volumes, the differences in the areas of CD31-positive vessels, between the SK-216–treated and untreated groups were observed in WT mice (Fig. 5C) but not in PAI-$1^{-/-}$ mice (Fig. 5D). In both WT and PAI-$1^{-/-}$ mice, there was no significant difference in the areas of CD31-positive vessels between subcutaneous tumors consisting of siControl LLC and siPAI-1 LLC cells (Fig. 5C and D).

SK-216 did not affect proliferation of HUVECs but inhibited migration and tube formation of HUVECs in vitro

On the basis of the inhibitory effect of SK-216 on angiogenesis in tumors of LLC and B16 cells *in vivo*, we assessed the *in vitro* effects of SK-216 on proliferation, migration, and tube formation of endothelial cells. Because production of VEGF in both LLC and B16 cells was confirmed (data not shown), the primary angiogenic factor in the tumors of LLC and B16 cells was thought to be VEGF. Therefore, we used VEGF to stimulate proliferation, migration, and tube formation of HUVECs in the *in vitro* assays. The proliferation assay showed that the presence of SK-216 at various concentrations in culture for 16 or 36 hours did not affect the cells' proliferation rates (Fig. 6A and B). As shown in Fig. 6C, however, the monolayer migration assay revealed that SK-216 inhibited the VEGF-induced migration of HUVECs in a dose-dependent manner. The statistically significant inhibition of HUVECs migration by SK-216 was observed at concentrations of 40 and 50 μmol/L. Furthermore, SK-216 was shown to inhibit VEGF-induced tube formation of HUVECs in a dose-dependent manner (Fig. 6E). The statistically significant inhibition of HUVECs tube formation by SK-216 was observed at concentrations of 30, 40, and 50 μmol/L.

Discussion

A growing body of evidence suggests that PAI-1 is closely involved in tumor progression and angiogenesis. In the present study, using a subcutaneous tumor model and a tail vein metastasis model, we have shown that systemic administration of SK-216, a specific PAI-1 inhibitor, was effective in suppressing both tumor progression and angiogenesis. This effect of SK-216 was found to be independent of the presence or absence of tumor PAI-1, suggesting the importance of host PAI-1 as a molecular target of SK-216. When the relevance for tumor progression

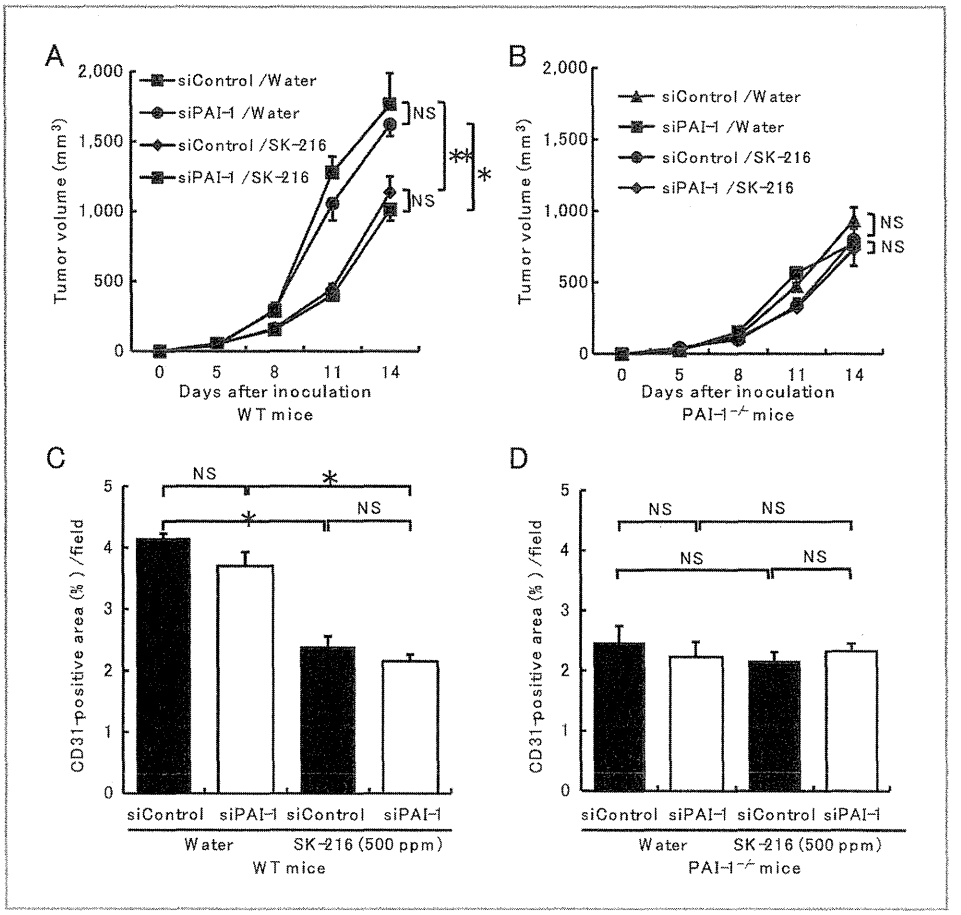

Figure 5. Influences of the presence or absence of host PAI-1 and the secreting levels of PAI-1 by tumor cells on effects of SK-216 on subcutaneous tumor growth and angiogenesis. A and B, evaluation of tumor sizes in the subcutaneous tumor model using siControl and siPAI-1 LLC cells. Volumes of subcutaneous tumors were measured twice a week for 2 weeks after inoculation of siControl or siPAI-1 LLC cells into WT (A) or PAI-1$^{-/-}$ (B) mice. Mice were given water or SK-216 (500 ppm). The data represent the mean values (±SEM) of 6 mice per group and were analyzed with the Student t test or Mann-Whitney U test as appropriate. *, $P < 0.01$; **, $P < 0.05$ versus control group. NS, not significant. C and D, evaluation of angiogenesis in subcutaneous tumors of WT (C) and PAI-1$^{-/-}$ (D) mice subcutaneously inoculated with siControl or siPAI-1 LLC cells. Mice were given water or SK-216 (500 ppm). The area of CD31-positive vessels was calculated as described in Materials and Methods. Data represent the mean values (±SEM) of 5 or 6 mice per group and were analyzed with the Student t test. *, $P < 0.05$ versus control group. NS, not significant.

and angiogenesis was compared between host and tumor PAI-1, we found that host but not tumor PAI-1 played a determinant role in these processes. These results also support the suggestion that SK-216 inhibited tumor progression and angiogenesis primarily through interacting with host PAI-1. In *in vitro* studies, SK-216 inhibited the VEGF-induced migration and tube formation of HUVECs.

There is only one previous study that used SK-216 for animal tumor models (26). In that study, SK-216 was shown to suppress the spontaneous formation of intestinal polyps in the adenomatous polyposis coli gene-deficient mouse. About another PAI-1 inhibitor, PAI-039, there is a report that it could reverse PAI-1's protection against apoptosis in human cancer cell lines (23). In the present study, we have shown the antitumor effect of SK-216 using a subcutaneous tumor model and a tail vein metastasis model. The most interesting finding was that the systemic administration of SK-216 could suppress tumor growth and lung metastasis irrespective of the presence or absence of PAI-1 secretion by the tumor cells. From the experiment that WT and PAI-1$^{-/-}$ mice subcutaneously inoculated with siPAI-1 LLC cells or siControl LLC cells were treated or untreated with SK-216, we also found that host but not tumor PAI-1 was determinant for the effect of SK-216 on tumor growth. These results suggest that the antitumor effect of SK-216 is likely exerted through interaction with host-derived PAI-1. In addition, in the present study, we have shown that the presence of host PAI-1 was a determinant in tumor growth and lung metastasis but the expression level of PAI-1 in tumor cells was not associated with either the degree of tumor growth or lung metastasis. Although we did not determine the precise mechanism by which host PAI-1 was involved in tumor progression, these results suggest that host PAI-1 was the primary molecular target for SK-216 in the animal tumor models used in the present study.

Although the crucial role of host PAI-1 in tumor progression has been reported (11–14), two recent studies showed the involvement of tumor PAI-1 in tumor growth. Nishioka and colleagues showed that reducing PAI-1 expression in either the tumor or the host could suppress tumor progression (22). In contrast, Fang and colleagues reported that both host PAI-1 and tumor PAI-1 had to be reduced to inhibit tumor progression (23). These two reports are inconsistent with our finding that the level of tumor PAI-1 did not affect the extent of tumor progression, and, unfortunately we do not have data to explain this difference. In animal tumor models using different tumor cells from those used in the present study, tumor PAI-1 might be associated with tumor progression. Because reduction of tumor PAI-1 expression or activity seemed to be advantageous for inhibiting tumor progression, we

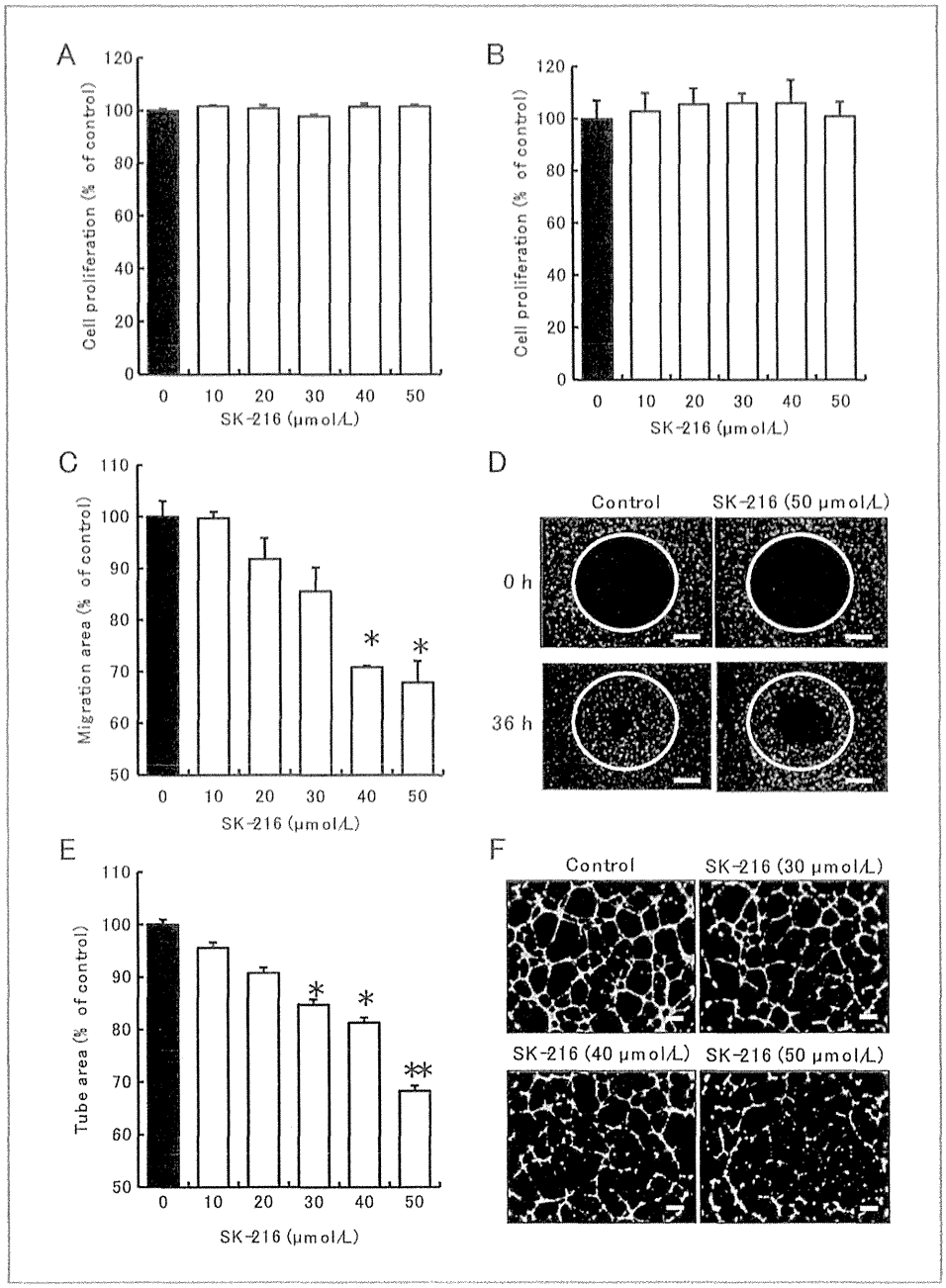

Figure 6. Effects of SK-216 on the degrees of proliferation, migration, and tube formation of HUVECs. A and B, proliferation of HUVECs induced by VEGF was quantified after 16 hours (A) and 36 hours (B) in the presence of SK-216 at various concentrations. C and E, migration (C) and tube formation (E) of HUVECs induced by VEGF in the presence of SK-216 at various concentrations were evaluated as described in Materials and Methods. D and F, representative images of HUVECs in cell migration assay (D) and capillary-like tube formation assay (F). HUVECs were stained with Calcein AM stock solution. Data represent the mean values (±SEM) of triplicate samples and were analyzed with the Student t test. *, $P < 0.05$ versus control; **, $P < 0.01$ versus control; scale bar, 250 μm.

believe that this difference should not be an obstacle to the use of SK-216 as a systemic antitumor agent.

We note that there was a study that was inconsistent with our results. Eitzman and colleagues reported that the expression level of host PAI-1 did not affect the extent of tumor growth in the foot pad or the formation of lung metastases by B16 cells (27). Unfortunately, we cannot readily explain this discrepancy. We speculate that differences between the sites where B16 cells were implanted and/or the number of cells used for experiments between Eitzman and colleagues' and our studies resulted in these inconsistent data.

Independent of tumor cells' expression of PAI-1, PAI-1 production is thought to be increased by soluble factors in the tumor microenvironment. It has been reported that VEGF produced by tumor cells and/or stromal host cells promoted PAI-1 secretion by endothelial cells (28). In addition, inflammatory cytokines such as interleukin (IL)-1, IL-6, and TNF-α from immune cells (29) and TGF-β from fibroblasts (30) are all known to induce PAI-1 expression in endothelial cells (31) and hepatocytes (32). Moreover, extravascular synthesis of PAI-1 by adipocytes (33), macrophages (34), and fibroblasts (35, 36) is promoted. Elevated levels of circulating PAI-1 in

tumor-bearing patients (37–39) seem to reflect overproduction of PAI-1 in the tumor environment. Considering the strong association between the abundance of PAI-1 in the tumor microenvironment and the aggressiveness of the tumor (6–9), systemic administration of SK-216 could be a reasonable therapeutic approach for the treatment of malignancy.

In the present study, the extent of angiogenesis in tumors generated in PAI-$1^{-/-}$ mice was significantly lower than that in WT mice. This result confirms the previous observations that indicated the significance of host PAI-1 in regulating tumor angiogenesis (11–14). Indeed, a previous study showed that PAI-1 produced by tumor cells, even at high concentrations, could not compensate for the absence of host PAI-1 in tumor angiogenesis (13). These observations suggest that host PAI-1 could become a novel molecular target for the reduction in tumor angiogenesis. Interestingly, the systemic administration of SK-216 reduced angiogenesis in tumors of PAI-1–secreting LLC cells and PAI-1–nonsecreting B16 cells, similar to that observed in PAI-$1^{-/-}$ mice. From the experiment that WT and PAI-$1^{-/-}$ mice subcutaneously inoculated with siPAI-1 LLC cells or siControl LLC cells were treated or untreated with SK-216, we also found that host but not tumor PAI-1 was determinant for the effect of SK-216 on angiogenesis. These results suggest that systemic administration of SK-216 reduced tumor angiogenesis through inhibition of host PAI-1 activity. In addition, the direct inhibitory effect of SK-216 on VEGF-mediated migration and tube formation of HUVECs was also shown in the present study. Although the precise mechanism of host PAI-1 involvement in tumor angiogenesis was not determined, these observations suggest that inhibition of host PAI-1 activity would result in the reduction of tumor angiogenesis, raising the possibility that systemic administration of SK-216 could become a novel antiangiogenic therapeutic in the treatment of malignancy.

Because the induction of angiogenesis is an important mechanism by which tumors promote their own continued growth and metastasis (40), inhibition of tumor angiogenesis represents an attractive therapeutic approach in the treatment of malignancy. VEGF plays a major role in tumor angiogenesis, however, the contribution of other factors, such as PDGF, FGF, and angiopoietins, has been confirmed (41–44). Currently, only VEGF-targeted antiangiogenic agents are clinically available for the treatment of malignancy. They include bevacizumab (Avastin; Genentech/Roche) targeting VEGF and two kinase inhibitors, sorafenib (Nexavar; Bayer) and sunitinib (Sutent; Pfizer), targeting the VEGF receptor signaling pathway. Thus, the development of antiangiogenic therapeutics with different targets seems necessary. The reduction of angiogenesis in the subcutaneous tumors of LLC and B16 cells by SK-216 raises the possibility that SK-216 could be used as an alternative antiangiogenic agent. The target of this antiangiogenic approach was found to be host-derived PAI-1. We believe that systemic administration of SK-216 proposes a new concept of antiangiogenic therapeutics that targets host-derived factors.

In conclusion, using systemic administration of SK-216, a specific inhibitor for PAI-1, we showed that it limited tumor progression and angiogenesis *in vivo*, independent of the presence or absence of PAI-1 secretion by the tumor cells. In addition, the results of the present study indicate that host (but not tumor) PAI-1 plays a determinant role in these processes. These results suggest the possibility that host PAI-1 was the main molecular target for SK-216. Furthermore, SK-216 was shown to have an inhibitory effect on migration and tube formation by HUVECs *in vitro*. Taken together, these observations strongly suggest that systemic administration of SK-216 reduced tumor progression mainly through its interaction with host PAI-1 and that this antitumor effect might be mediated by the antiangiogenic properties of SK-216.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Development of methodology: T. Masuda, T. Senoo

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Masuda, T. Senoo, S. Akita

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Masuda, T. Senoo

Writing, review, and/or revision of the manuscript: T. Masuda, N. Hattori, T. Senoo, N. Ishikawa, K. Fujitaka, Y. Haruta, H. Murai, N. Kohno

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Senoo, S. Akita

Study supervision: N. Hattori, T. Senoo, K. Fujitaka, N. Kohno

Grant Support

This study was supported by grants-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (no. 22390165; N. Hattori).

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Received January 16, 2013; revised August 16, 2013; accepted August 19, 2013; published OnlineFirst August 29, 2013.

References

1. Rakic JM, Maillard C, Jost M, Bajou K, Masson V, Devy L, et al. Role of plasminogen activator-plasmin system in tumor angiogenesis. Cell Mol Life Sci 2003;60:463-73.
2. Smith HW, Marshall CJ. Regulation of cell signalling by uPAR. Nat Rev Mol Cell Biol 2010;11:23-36.
3. Stefansson S, Petitclerc E, Wong MK, McMahon GA, Brooks PC, Lawrence DA. Inhibition of angiogenesis in vivo by plasminogen activator inhibitor-1. J Biol Chem 2001;276:8135-41.
4. Konecny G, Untch M, Pihan A, Kimmig R, Gropp M, Stieber P, et al. Association of urokinase-type plasminogen activator and its inhibitor

with disease progression and prognosis in ovarian cancer. Clin Cancer Res 2001;7:1743-9.
5. Heiss MM, Allgayer H, Gruetzner KU, Babic R, Jauch KW, Schildberg FW. Clinical value of extended biologic staging by bone marrow micrometastases and tumor-associated proteases in gastric cancer. Ann Surg 1997;226:736-44
6. Foekens JA, Peters HA, Look MP, Portengen H, Schmitt M, Kramer MD, et al. The urokinase system of plasminogen activation and prognosis in 2780 breast cancer patients. Cancer Res 2000;60:636-43.
7. Sakakibara T, Hibi K, Kodera Y, Ito K, Akiyama S, Nakao A. Plasminogen activator inhibitor-1 as a potential marker for the malignancy of esophageal squamous cell carcinoma. Clin Cancer Res 2004;10: 1375-8.
8. Muracciole X, Romain S, Dufour H, Palmari J, Chinot O, Ouafik L, et al. PAI-1 and EGFR expression in adult glioma tumors: toward a molecular prognostic classification. Int J Radiat Oncol Biol Phys 2002;52: 592-8.
9. Ohba K, Miyata Y, Kanda S, Koga S, Hayashi T, Kanetake H. Expression of urokinase-type plasminogen activator, urokinase-type plasminogen activator receptor and plasminogen activator inhibitors in patients with renal cell carcinoma: correlation with tumor associated macrophage and prognosis. J Urol 2005;174:461-5.
10. Werle B, Kotzsch M, Lah TT, Kos J, Gabrijelcic-Geiger D, Spiess E, et al. Cathepsin B, plasminogenactivator-inhibitor (PAI-1) and plasminogen activator-receptor (uPAR) are prognostic factors for patients with non-small cell lung cancer. Anticancer Res 2004;24:4147-61.
11. Bajou K, Noel A, Gerard RD, Masson V, Brunner N, Holst-Hansen C, et al. Absence of host plasminogen activator inhibitor 1 prevents cancer invasion and vascularization. Nat Med 1998;4:923-8.
12. Gutierrez LS, Schulman A, Brito-Robinson T, Noria F, Ploplis VA, Castellino FJ. Tumor development is retarded in mice lacking the gene for urokinase-type plasminogen activator or its inhibitor, plasminogen activator inhibitor-1. Cancer Res 2000;60:5839-47.
13. Bajou K, Maillard C, Jost M, Lijnen RH, Gils A, Declerck P, et al. Host-derived plasminogen activator inhibitor-1 (PAI-1) concentration is critical for in vivo tumoral angiogenesis and growth. Oncogene 2004;23:6986-90.
14. Maillard C, Jost M, Romer MU, Brunnery N, Houard X, Lejeune A, et al. Host plasminogen activator inhibitor-1 promotes human skin carcinoma progression in a stage-dependent manner. Neoplasia 2005;7:57-66.
15. Bajou K, Masson V, Gerard RD, Schmitt PM, Albert V, Praus M, et al. The plasminogen activator inhibitor PAI-1 controls in vivo tumor vascularization by interaction with proteases, not vitronectin. Implications for antiangiogenic strategies. J Cell Biol 2001;152:777-84.
16. Devy L, Blacher S, Grignet-Debrus C, Bajou K, Masson V, Gerard RD, et al. The pro- or antiangiogenic effect of plasminogen activator inhibitor 1 is dose dependent. FASEB J 2002;16:147-54.
17. Waltz DA, Natkin LR, Fujita RM, Wei Y, Chapman HA. Plasmin and plasminogen activator inhibitor type 1 promote cellular motility by regulating the interaction between the urokinase receptor and vitronectin. J Clin Invest 1997;100:58-67.
18. Loskutoff DJ, Curriden SA, Hu G, Deng G. Regulation of cell adhesion by PAI-1. APMIS 1999;107:54-61.
19. Bajou K, Peng H, Laug WE, Maillard C, Noel A, Foidart JM, et al. Plasminogen activator inhibitor-1 protects endothelial cells from FasL-mediated apoptosis. Cancer Cell 2008;14:324-34.
20. Romer MU, Larsen L, Offenberg H, Brunner N, Lademann UA. Plasminogen activator inhibitor 1 protects fibrosarcoma cells from etoposide-induced apoptosis through activation of the PI3K/Akt cell survival pathway. Neoplasia 2008;10:1083-91.
21. Kwaan HC, Wang J, Svoboda K, Declerck PJ. Plasminogen activator inhibitor 1 may promote tumour growth through inhibition of apoptosis. Br J Cancer 2000;82:1702-8.
22. Nishioka N, Matsuoka T, Yashiro M, Hirakawa K, Olden K, Roberts JD. Plasminogen activator inhibitor 1 RNAi suppresses gastric cancer metastasis in vivo. Cancer Sci 2012;103:228-32.
23. Fang H, Placencio VR, Declerck YA. Protumorigenic activity of plasminogen activator inhibitor-1 through an antiapoptotic function. J Natl Cancer Inst 2012;104:1470-84.
24. Charlton PA, Faint RW, Bent F, Bryans J, Chicarelli-Robinson I, Mackie I, et al. Evaluation of a low molecular weight modulator of human plasminogen activator inhibitor-1 activity. Thromb Haemost 1996;75: 808-15.
25. Worzalla JF, Bewley JR, Grindey GB. Automated measurement of transplantable solid tumors using digital electronic calipers interfaced to a microcomputer. Invest New Drugs 1990;8:241-51.
26. Mutoh M, Niho N, Komiya M, Takahashi M, Ohtsubo R, Nakatogawa K, et al. Plasminogen activator inhibitor-1 (Pai-1) blockers suppress intestinal polyp formation in Min mice. Carcinogenesis 2008;29: 824-9.
27. Eitzman DT, Krauss JC, Shen T, Cui J, Ginsburg . Lack of plasminogen activator inhibitor-1 effect in a transgenic mouse model of metastatic melanoma. Blood 1996;87:4718-22.
28. Olofsson B, Korpelainen E, Pepper MS, Mandriota SJ, Aase K, Kumar V, et al. Vascular endothelial growth factor B (VEGF-B) binds to VEGF receptor-1 and regulates plasminogen activator activity in endothelial cells. Proc Natl Acad Sci U S A 1998;95:11709-14
29. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell 2010;140:883-99.
30. Nakagawa H, Liyanarachchi S, Davuluri RV, Auer H, Martin EW Jr, de la Chapelle A, et al. Role of cancer-associated stromal fibroblasts in metastatic colon cancer to the liver and their expression profiles. Oncogene 2004;23:7366-77.
31. Sawdey M, Podor TJ, Loskutoff DJ. Regulation of type 1 plasminogen activator inhibitor gene expression in cultured bovine aortic endothelial cells. Induction by transforming growth factor-beta, lipopolysaccharide, and tumor necrosis factor-alpha. J Biol Chem 1989;264:10396-401.
32. Seki T, Healy AM, Fletcher DS, Noguchi T, Gelehrter TD. IL-1beta mediates induction of hepatic type 1 plasminogen activator inhibitor in response to local tissue injury. Am J Physiol 1999;277:801-9.
33. Loskutoff DJ, Samad F. The adipocyte and hemostatic balance in obesity: studies of PAI-1. Arterioscler Thromb Vasc Biol 1998;18: 1-6.
34. Chapman HA, Yang XL, Sailor LZ, Sugarbaker DJ. Developmental expression of plasminogen activator inhibitor type 1 by human alveolar macrophages. Possible role in lung injury. J Immunol 1990; 145:3398-405.
35. Lund LR, Riccio A, Andreasen PA, Nielsen LS, Kristensen P, Laiho M, et al. Transforming growth factor-beta is a strong and fast acting positive regulator of the level of type-1 plasminogen activator inhibitor mRNA in WI-38 human lung fibroblasts. EMBO J 1987;6: 1281-6.
36. Samad F, Bergtrom G, Amrani DL. Regulation of plasminogen activation by interleukin-6 in human lung fibroblasts. Biochim Biophys Acta 1994;1221:307-14.
37. Ho CH, Chao Y, Lee SD, Chau WK, Wu CW, Liu SM. Diagnostic and prognostic values of plasma levels of fibrinolytic markers in gastric cancer. Thromb Res 1998;91:23-7.
38. Ho CH, Yuan CC, Liu SM. Diagnostic and prognostic values of plasma levels of fibrinolytic markers in ovarian cancer. Gynecol Oncol 1999;75:397-400.
39. Sciacca FL, Ciusani E, Silvani A, Corsini E, Frigerio S, Pogliani S, et al. Genetic and plasma markers of venous thromboembolism in patients with high grade glioma. Clin Cancer Res 2004;10:1312-7.
40. Folkman J. What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 1990;82:4-6.
41. Erber R, Thurnher A, Katsen AD, Groth G, Kerger H, Hammes HP, et al. Combined inhibition of VEGF and PDGF signaling enforces tumor vessel regression by interfering with pericyte-mediated endothelial cell survival mechanisms. FASEB J 2004;18:338-40.
42. Compagni A, Wilgenbus P, Impagnatiello MA, Cotten M, Christofori G. Fibroblast growth factors are required for efficient tumor angiogenesis. Cancer Res 2000;60:7163-9.
43. Holash J, Maisonpierre PC, Compton D, Boland P, Alexander CR, Zagzag D, et al. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science 1999;284:1994-8.
44. Kerbel RS. Tumor angiogenesis. N Engl J Med 2008;358:2039-49.

Official Journal of the Asian Pacific Society of Respirology
Respirology

ORIGINAL ARTICLE

Prognostic significance of fibroblastic foci in usual interstitial pneumonia and non-specific interstitial pneumonia

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ABSTRACT

Background and objective: **Fibroblastic foci (FF) composed of an accumulation of fibroblasts or myofibroblasts may be related to the progression of pulmonary fibrosis leading to respiratory insufficiency. Several studies have shown that the number of FF is a significant prognostic factor in usual interstitial pneumonia (UIP). The purpose of the present study was to examine whether the extent of FF is related to impairment of respiratory function and prognosis in patients with biopsy-proven fibrosing interstitial pneumonia, including UIP and fibrotic non-specific interstitial pneumonia (fNSIP).**

Methods: **Fifty patients with histologically confirmed interstitial pneumonia including UIP or fNSIP were investigated, and correlations between FF and pulmonary function were evaluated. FF area was calculated as the proportion of total area (%FF) and the number of FF (FF/cm^2) in the whole histological specimen from each patient.**

Results: **The UIP group showed significantly higher %FF and FF/cm^2 than the fNSIP group. When UIP and fNSIP patients were analysed together, the group of patients who had died (death group) revealed significantly higher %FF and FF/cm^2 compared with the group of survivors, and the impairment of vital capacity and diffusing capacity of carbon monoxide was correlated with %FF and FF/cm^2.**

Conclusions: **FF correlated with impaired pulmonary function and may be a useful parameter to predict prognosis in patients with UIP and fNSIP.**

Key words: fibroblastic focus, non-specific interstitial pneumonia, pulmonary function, usual interstitial pneumonia.

Abbreviations: DL_{CO}, diffusing capacity of carbon monoxide; FF, fibroblastic foci; fNSIP, fibrotic non-specific interstitial pneumonia; UIP, usual interstitial pneumonia; VC, vital capacity.

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Received 23 December 2011; invited to revise 23 February 2012, 28 August 2012; revised 18 May 2012; accepted 4 September 2012 (Associate Editor: Yuben Moodley).

SUMMARY AT A GLANCE

A significant correlation was demonstrated between histological findings and respiratory function in fibrosing interstitial pneumonia. Our findings indicate that fibroblastic foci are a reliable predictor of prognosis in usual interstitial pneumonia and fibrotic non-specific interstitial pneumonia.

INTRODUCTION

Fibroblastic foci (FF) are characteristic features of usual interstitial pneumonia (UIP) and are associated with progression of disease and a poor prognosis.[1–3] They are composed of aggregates of mesenchymal cells including fibroblasts and myofibroblasts and may play a critical role in the progression of pulmonary fibrosis.

There has been an interest in the prognostic significance of FF in UIP. Several studies have demonstrated the clinical importance of FF as a prognostic parameter, establishing that poor prognosis is related to a greater number of FF.[1–3] However, some studies have failed to demonstrate the correlation between FF and survival in patients with idiopathic pulmonary fibrosis, indicating that quantitative assessment of FF in surgical lung biopsy specimens is of limited prognostic value.[4–6] These conflicting results are probably a result of the different methods used to select samples from patients. In the previous studies, FF was examined only in UIP, not in non-specific interstitial pneumonia. Furthermore, previous studies analysed FF in selected areas of lung specimens using a score grading system, not in the whole specimen.[7] In addition, a recent guideline indicates that surgical lung biopsy is not essential in the subjects showing UIP pattern on high-resolution computed tomography. As a result, biopsy would be performed only in patients with the difficulty in computed tomography diagnosis. This could cause a difficulty to distinguish histologically between UIP and fibrotic non-specific interstitial pneumonia (fNSIP). The purpose of the

doi: 10.1111/j.1440-1843.2012.02272.x

present study was to examine whether the extent of FF is related to impairment of respiratory function and prognosis in patients with biopsy-proven fibrosing interstitial pneumonia, including UIP and fNSIP.

METHODS

Patients

We reviewed patients with interstitial pneumonia who had open or a video-assisted thoracoscopic lung biopsy in Fukuoka University Hospital, National Hospital Organization Kyushu Medical Center, and Fukuoka National Hospital between 1995 and 2008. Fifty patients with histologically confirmed UIP or fNSIP were selected. Patients with concomitant lung neoplasms were excluded. Histological diagnosis of UIP and fNSIP was based on a previously published report[8] and the criteria in the American Thoracic Society/European Respiratory Society consensus classification.[9] The study protocol was approved by the ethics committee of our institute.

Pathological evaluation

The specimens were routinely fixed in 10% formalin and were processed into paraffin blocks for histopathological examination. Tissue sections were cut 4 μm thick and stained with HE, Alcian blue-periodic acid Schiff and elastica van Gieson.

FF, newly formed connective tissue bundles, were defined as subepithelial, interstitial areas in which fibroblasts and myofibroblasts were arranged in a linear manner within a pale staining matrix and which were stained blue by Alcian blue-periodic acid Schiff (Fig. 1).

The area of FF and the whole lung tissue area were measured using image analysis software (WinROOF version 5.5, Mitani Corporation, Fukui, Japan). The total number of FF was obtained by counting all FF in all specimens from each patient, and FF/cm^2 was calculated by dividing the total number of FF by the whole lung tissue area (cm^2). %FF was calculated by dividing the total area of FF by the whole lung tissue area.

Respiratory function data

All respiratory function data, including vital capacity (VC) and diffusing capacity (diffusing capacity of carbon monoxide (DL_{CO})) during the entire follow-up period, were obtained from the medical records. Annual changes in respiratory function were estimated from linear regressions, assuming time dependency and linearity. Percentage decrease from the baseline per year (%ΔVC, %ΔDL_{CO}) was obtained from the linear equation, and the correlation between the respiratory function impairment and FF was evaluated.

Statistical analysis

Numerical data are presented as the mean ± standard error of the mean. For analysis, the unpaired Student's *t*-test was used for pairwise comparisons. Categorical data were compared between UIP and fNSIP using the chi-square test for independence. Prognostic data were analysed using the Kaplan–Meier curve with log–rank test. Correlations involving FF and respiratory function data were evaluated using the Spearman rank coefficient. Statistical analysis was performed with StatView software for Windows version 5.0 (SAS Institute Inc., Cary, NC, USA). *P*-values less than 0.05 were considered significant.

RESULTS

Clinical characteristics, laboratory and physiological findings

The patient population consisted of 28 males and 22 females; mean age was 61.2 years. Of the 50 patients, 24 (48.0%) were diagnosed with UIP and 26 (51.0%)

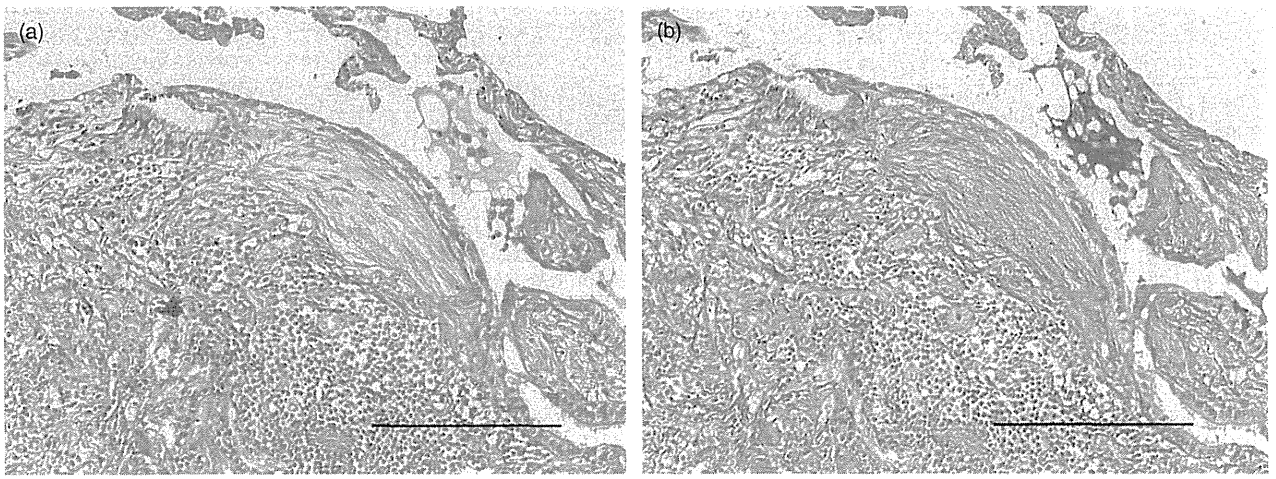

Figure 1 A histopathological example of fibroblastic foci. (a) HE section. Fibroblastic foci are composed of aggregates of fibroblasts or myofibroblasts arranged in a linear manner within a pale staining matrix. Scale bars = 200μm. Original magnification × 200. (b) Alcian blue-periodic acid Schiff (AB-PAS) section. Matrix of fibroblastic foci is stained blue. Scale bars = 200μm. Original magnification × 200.

Table 1 Clinical characteristics, and laboratory and physiological findings of the study population

Variables	UIP	NSIP	*P*-value
Clinical characteristics			
Male/female gender, No.	15/9	13/13	0.3737
Age at biopsy, year	65.3 ± 9.8	57.4 ± 11.5	0.0120*
Pack years of smoking	18.4 ± 28.9	19.0 ± 30.0	0.9476
Month since onset of symptoms	24.3 ± 37.8	12.3 ± 16.0	0.2064
Laboratory findings			
Serum KL-6, U/mL	1075 ± 615	2190 ± 2263	0.0423*
Serum LDH, IU	298 ± 191	286 ± 121	0.7930
Physiological findings			
VC, %predicted	72.5 ± 18.8	85.5 ± 16.2	0.0387*
DL_{CO}, %predicted	70.6 ± 23.4	82.9 ± 20.3	0.1261
PaO_2 at room air, torr	81.5 ± 14.1	83.5 ± 11.1	0.6181

* $P < 0.05$.

DL_{CO}, diffusing capacity of carbon monoxide; KL-6, Krebs von den Lungen-6; LDH, lactate dehydrogenase; NSIP, non-specific interstitial pneumonia; PaO_2, partial pressure of oxygen (arterial); UIP, usual interstitial pneumonia; VC, vital capacity.

with fNSIP. The clinical characteristics and laboratory findings of the patients are summarized in Table 1.

Of the clinical characteristics, age at lung biopsy was significantly different between UIP and fNSIP. Of 50 cases, 9 were current smokers, 13 were ex-smokers and 28 were never smokers. Serum Krebs von den Lungen-6 levels differed significantly between UIP and fNSIP groups (UIP < fNSIP).

Collagen vascular disease was found in five cases from the UIP group and four cases from the fNSIP group during the follow-up period.

Quantitative analysis of FF: Comparison between UIP and fNSIP, and between death and survival groups

Of 50 cases in our study, 9 cases had one biopsy sample and 41 patients had multiple biopsy samples. We divided all the samples into four groups based on biopsied sites (i.e. upper lobe, middle lobe, lingula and lower lobe). Nineteen per cent were obtained from upper lobe, 21% from middle lobe, 12% from lingula and 48% from lower lobe.

In UIP patients, %FF (0.266 ± 0.233) and FF/cm^2 (7.07 ± 45.12) were significantly higher than in fNSIP patients: %FF (0.045 ± 0.054) ($P < 0.0001$, Fig. 2a) and FF/cm^2 (1.69 ± 2.07) ($P < 0.0001$, Fig. 2b).

We investigated the area of normal lung, fibrosis and honeycomb lung by reviewing the available samples of 30 patients. In fNSIP, the area of normal lung to fibrosis was 40.7 ± 29.4% and 59.3 ± 29.4%, respectively, and in UIP, 36.5 ± 34.3% and 63.5 ± 34.3%, respectively (not significant). Among fibrosis, the honeycombing area was 4.8 ± 10.3% in fNSIP and 24.6 ± 24.7% in UIP, a significant difference ($P = 0.0284$).

Since follow up was done in all patients, we divided these into those who died (death group, $n = 13$) and those who survived (survival group, $n = 37$) and compared %FF and FF/cm^2 between the groups. The mean follow-up period for the survival group and the mean survival period for the death group were 55.2 months and 33.6 months, respectively. %FF in the death group (0.292 ± 0.259) was significantly higher than in the survival group (0.101 ± 0.106) ($P = 0.0020$, Fig. 3a). FF/cm^2 in the death group (7.45 ± 6.17) was also higher than in the survival group (3.16 ± 3.49) ($P = 0.0034$, Fig. 3b). Significant differences in %FF and FF/cm^2 between the two groups were also demonstrated when only UIP patients were analysed, but results for fNSIP patients only showed no significance (data not shown).

We reviewed the prognosis and clinical course for all patients enrolled in this study to draw the Kaplan–Meier curve. Significant prognostic difference between UIP and fNSIP was demonstrated by log–rank test ($P = 0.0233$, Fig. 4).

Correlation between FF and respiratory function parameters

The initial value of %VC has an inverse correlation with FF/cm^2 when UIP and fNSIP patients were analysed together ($rs = 0.211$, Fig. 5a). Also, as shown in Figure 5b,c, %ΔVC was correlated with both %FF and FF/cm^2 ($rs = 0.333$ and 0.294). $\%\Delta DL_{CO}$ was correlated only with %FF ($rs = 0.268$, Fig. 5d). However, the initial value of $\%DL_{CO}$ did not have any obvious correlation with FF. When the UIP and fNSIP patients were analysed separately, no significant correlations were observed (data not shown).

DISCUSSION

This is the first study to examine the area and the number of FF in both UIP and fNSIP samples obtained from surgical lung biopsy. Based on the assumption that FF is a common histological finding in both UIP and fNSIP that reflects disease progression, we examined the relationship between the extent of FF and the respiratory function in a combined group of patients with UIP or fNSIP. A correlation between FF and both respiratory function and

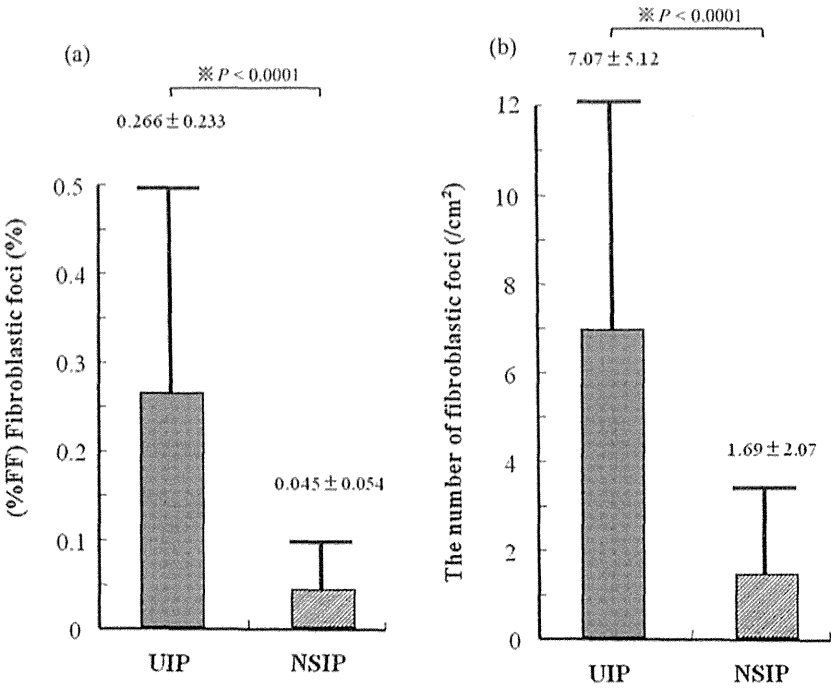

Figure 2 Quantitative analysis of fibroblastic foci in the whole specimen. (a) The areal percentage of fibroblastic foci was significantly higher in usual interstitial pneumonia (UIP) group than in non-specific interstitial pneumonia (NSIP) group. (b) The number of fibroblastic foci was significantly higher in UIP group than in NSIP group. Data are mean ± standard deviation.

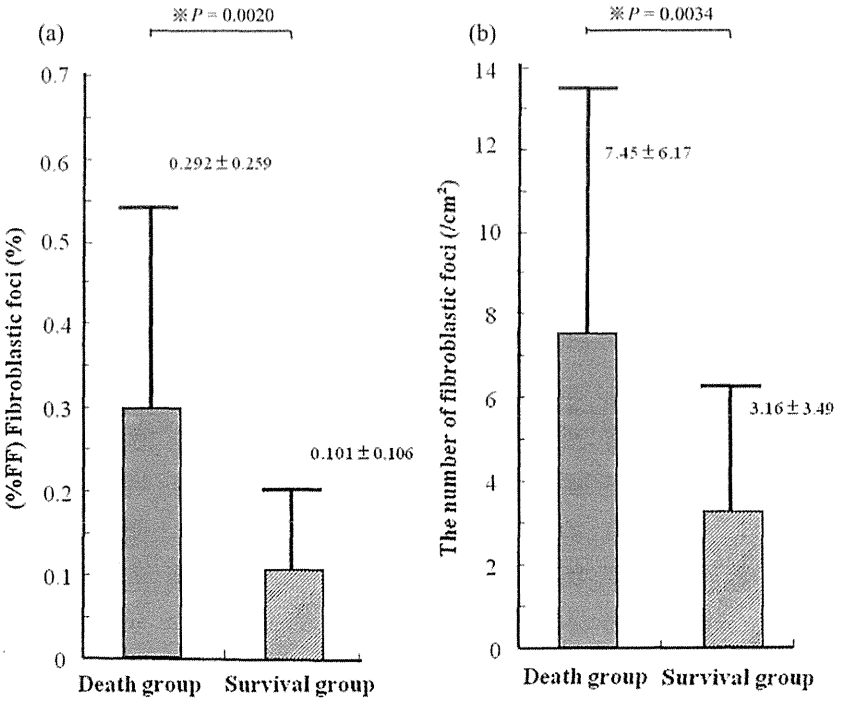

Figure 3 Quantitative analysis of fibroblastic foci of all cases comparing death group and survival group. (a) The areal percentage of fibroblastic foci was significantly higher in death group than in survival group. (b) The number of fibroblastic foci was significantly higher in death group than in survival group. Data are mean ± standard deviation.

prognosis was demonstrated. Our result revealed that the decline in VC and DL_{CO} was significantly correlated with the increasing area and/or number of FF. Furthermore, the group of patients who died had a higher degree of FF compared with the survival group.

There have been various attempts to relate disease progression to the extent of FF.[1–7] Most of these studies were performed using UIP samples. However, fNSIP accepts some temporal range in lung injury, allowing the presence of some FF coexistent with old fibrosis (e.g. honeycombing).[9–11] These ambiguous criteria might be confusing when making a differentiation between UIP and fNSIP. Furthermore, to what extent FF are considered to exist in the case of an fNSIP pattern remains unclear.

Practically, lung biopsy tends to be performed in patients whose computed tomography patterns are non-UIP-like, and patients with computed tomogra-

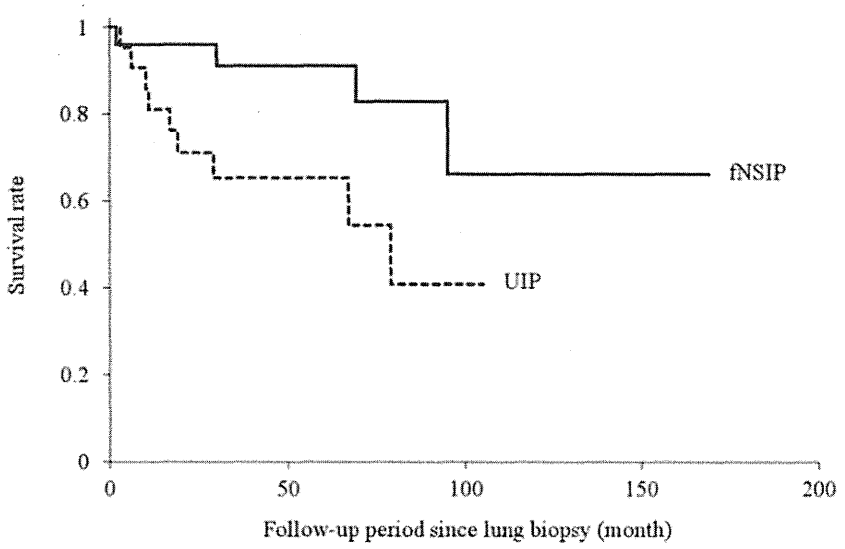

Figure 4 Kaplan–Meier curve of patients enrolled in the study. Patients with fibrotic non-specific interstitial pneumonia (fNSIP) survived significantly longer than those with usual interstitial pneumonia (UIP) by log–rank test ($P = 0.0233$).

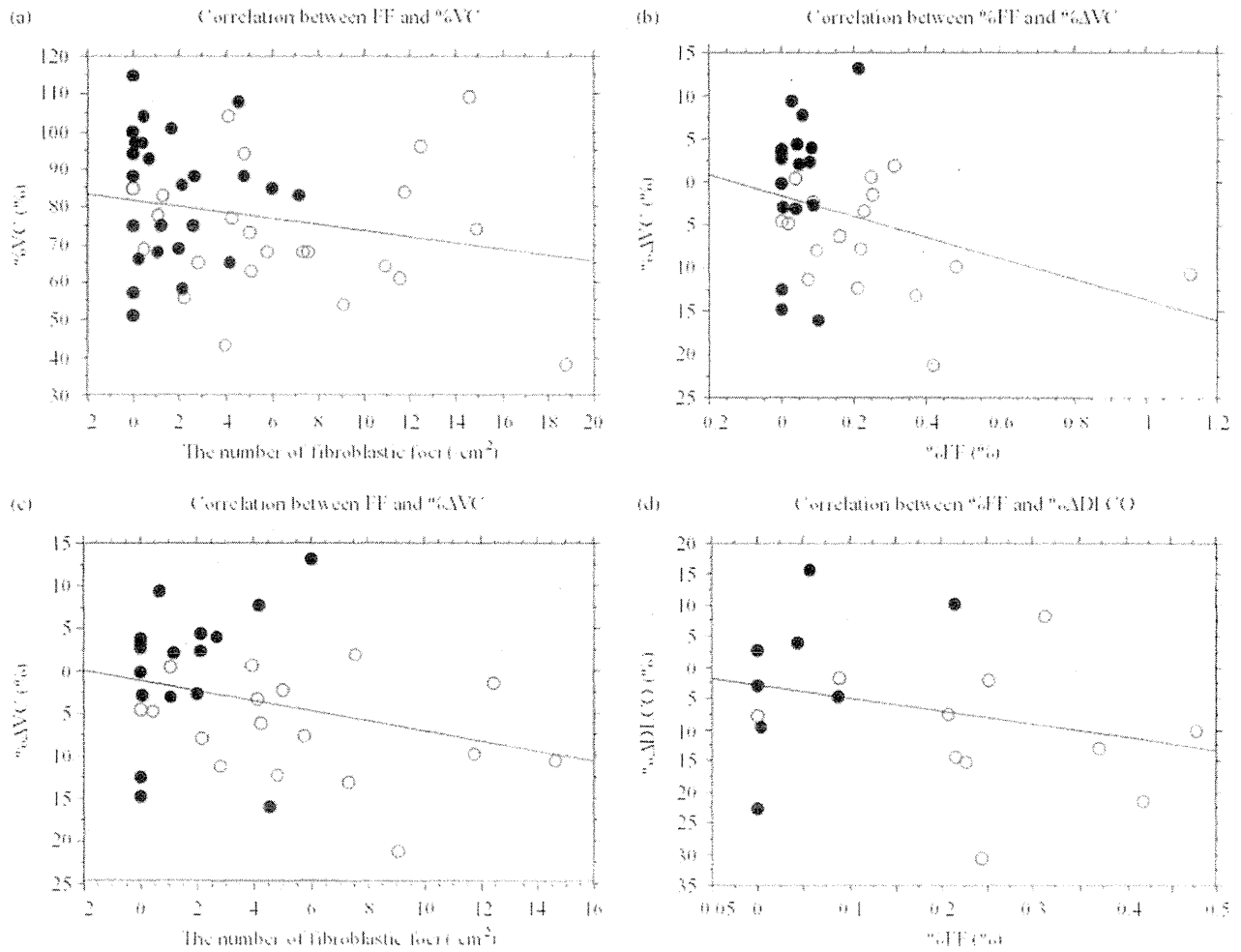

Figure 5 Correlation between pulmonary function and fibroblastic foci (FF). %FF and the number of FF correlated inversely with (a) % vital capacity (VC) and (b,c) %ΔVC (a: $rs = 0.211$; b: $rs = 0.333$; c: $rs = 0.294$); (d) %Δ diffusing capacity of carbon monoxide correlated only with %FF ($rs = 0.268$). (●) fNSIP, fibrotic non-specific interstitial pneumonia; (○) UIP, usual interstitial pneumonia.

phy patterns typical of UIP rarely have the opportunity to receive a lung biopsy. This may cause difficulty in histologically categorizing interstitial pneumonia. Furthermore, in our fNSIP patients, the time between onset of symptoms and the visit to the hospital was longer than previously reported.[12] It is therefore possible that some cases classified as fNSIP in our study could be diagnosed as idiopathic pulmonary fibrosis after consensus clinical, radiological and pathological review. According to the report of an American Thoracic Society project,[13] a high-resolution computed tomography showing a pattern typical of UIP, such as honeycombing in the subpleural areas of the bilateral lower lobes, could lead to a diagnosis of idiopathic pulmonary fibrosis even when a surgical lung biopsy shows the histological pattern of fNSIP.

Latsi and co-workers[14] analysed the prognosis of idiopathic fibrosing interstitial pneumonia, demonstrating that the distinction between UIP and fNSIP provides no additional prognostic information once serial pulmonary function trends have been taken into account at 12-month follow up. Furthermore, when the initial DL_{CO} was less than 35% predicted, there was no significant difference in outcome between UIP and fNSIP, suggesting that the pathological pattern is less important for prognosis in the setting of relatively severe impairment of lung function. Similarly, patients who exhibit more than 10% decrease in forced VC have a poor outcome, whether they are classified as UIP or fNSIP.[15] These data show the role of respiratory function in addition to histological classification as a prognostic indicator. Our results indicate the significance of FF related to deteriorating pulmonary function in fibrosing interstitial pneumonia consisting of UIP and fNSIP.

It is also known that FF represent a measure of UIP activity, being associated with a poor prognosis, and several studies demonstrate the clinical importance of FF as a prognostic factor.[1–3] Nicholson and co-workers[3] demonstrated that an increasing semiquantitative FF score was independently associated with greater declines in forced VC and DL_{CO} at both 6 months and 12 months. King and co-workers[2] demonstrated an association between increasing FF, including the results of semiquantitative grading of the number of FF, and decreased survival in UIP. These data suggest that quantifying the number of these lesions in a biopsy specimen provides additional prognostic information. Moreover, Enomoto and co-workers[7] reported quantitative analysis of FF in UIP, showing that the quantitative fibroblast score was a highly significant predictor of outcome in combined analysis of patients with idiopathic pulmonary fibrosis and those with collagen vascular disease. Our results are almost entirely in agreement with these previous investigations. However, the %FF in this study is definitely lower, even in UIP samples, than that reported by Enomoto *et al.* Their analysis differed from ours in that 10 randomly selected fields were chosen for analysis. These differences in methods for selecting microscopic fields for study may cause the difference in the %FF data.

We have provided evidence of a correlation between FF and pulmonary function in both UIP and fNSIP patients. This result indicates that FF may be another factor predicting the prognosis of fibrosing interstitial pneumonias including both UIP and fNSIP. Increasing degrees of FF and the decline of forced VC or DL_{CO} might be important prognostic predictors for fNSIP as well as for UIP. Given the poor benefit achieved with immunosuppressive or anti-inflammatory agents, these data suggest that future therapies should be aimed at preventing or inhibiting the fibroproliferative response.

Acknowledgement

This work was partly supported by a grant to the Diffuse Lung Diseases Research Group from the Ministry of Health, Labour and Welfare, Japan.

REFERENCES

1 King TE Jr, Tooze JA, Schwarz MI *et al.* Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. *Am. J. Respir. Crit. Care Med.* 2001; **164**: 1171–81.
2 King TE Jr, Schwarz MI, Brown K *et al.* Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. *Am. J. Respir. Crit. Care Med.* 2001; **164**: 1025–32.
3 Nicholson AG, Fulford LG, Colby TV *et al.* The relationship between individual histologic features and disease progression in idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 2002; **166**: 173–7.
4 Collard HR, Cool CD, Leslie KO *et al.* Organizing pneumonia and lymphoplasmacytic inflammation predict treatment response in idiopathic pulmonary fibrosis. *Histopathology* 2007; **50**: 258–65.
5 Hanak V, Ryu JH, de Carvalho E *et al.* Profusion of fibroblast foci in patients with idiopathic pulmonary fibrosis does not predict outcome. *Respir. Med.* 2008; **102**: 852–6.
6 Flaherty KR, Colby TV, Travis WD *et al.* Fibroblastic foci in usual interstitial pneumonia: idiopathic versus collagen vascular disease. *Am. J. Respir. Crit. Care Med.* 2003; **167**: 1410–5.
7 Enomoto N, Suda T, Kato M *et al.* Quantitative analysis of fibroblastic foci in usual interstitial pneumonia. *Chest* 2006; **130**: 22–9.
8 Katzenstein AL, Myers JL. Idiopathic pulmonary fibrosis: clinical relevance of pathologic classification. *Am. J. Respir. Crit. Care Med.* 1998; **157**: 1301–15.
9 American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. *Am. J. Respir. Crit. Care Med.* 2002; **165**: 277–304.
10 Katzenstein AL, Fiorelli RF. Nonspecific interstitial pneumonia/fibrosis. Histologic features and clinical significance. *Am. J. Surg. Pathol.* 1994; **18**: 136–47.
11 Flaherty KR, Martinez FJ, Travis W *et al.* Nonspecific interstitial pneumonia (NSIP). *Semin. Respir. Crit. Care Med.* 2001; **22**: 423–34.
12 Nagai S, Kitaichi M, Itoh H *et al.* Idiopathic nonspecific interstitial pneumonia/fibrosis: comparison with idiopathic pulmonary fibrosis and BOOP. *Eur. Respir. J.* 1998; **12**: 1010–9.
13 Travis WD, Hunninghake G, King TE Jr *et al.* Idiopathic nonspecific interstitial pneumonia. Report of an American Thoracic Society project. *Am. J. Respir. Crit. Care. Med.* 2008; **177**: 1338–47.
14 Latsi PI, du Bois RM, Nicholson AG *et al.* Fibrotic idiopathic interstitial pneumonia: the prognostic value of longitudinal functional trends. *Am. J. Respir. Crit. Care Med.* 2003; **168**: 531–7.
15 Jegal Y, Kim DS, Shim TS *et al.* Physiology is a stronger predictor of survival than pathology in fibrotic interstitial pneumonia. *Am. J. Respir. Crit. Care Med.* 2005; **171**: 639–44.

suggests that neutrophilic airway inflammation may be one such factor although this relationship may be a consequence of coughing rather than causal.

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Statement of Interest: Statements of interest for S. Birring and I. Pavord can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

REFERENCES

1 Pavord ID, Chung KF. Management of chronic cough. *Lancet* 2008; 371: 1375–1384.
2 Morice AH, McGarvey L, Pavord ID. Recommendations for the management of cough in adults. *Thorax* 2006; 61: 1–24.
3 Birring SS, Fleming T, Matos S, *et al.* The Leicester Cough Monitor: preliminary validation of an automated cough detection system in chronic cough. *Eur Respir J* 2008; 31: 1013–1018.
4 Matos S, Birring SS, Pavord ID, *et al.* Detection of cough signals in continuous audio recordings using hidden Markov models. *IEEE Trans Biomed Eng* 2006; 53: 1078–1083.
5 Prudon B, Birring SS, Vara DD, *et al.* Cough and glottic-stop reflex sensitivity in health and disease. *Chest* 2005; 127: 550–557.
6 Birring SS, Matos S, Patel RB, *et al.* Cough frequency, cough sensitivity and health status in patients with chronic cough. *Respir Med* 2006; 100: 1105–1109.
7 Pavord ID, Pizzichini MM, Pizzichini E, *et al.* The use of induced sputum to investigate airway inflammation. *Thorax* 1997; 52: 498–501.
8 Birring SS, Prudon B, Carr AJ, *et al.* Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ). *Thorax* 2003; 58: 339–343.
9 Morice AH, Lowry R, Brown MJ, *et al.* Angiotensin-converting enzyme and the cough reflex. *Lancet* 1987; 2: 1116–1118.

DOI: 10.1183/09031936.00089312

Pleuroparenchymal fibroelastosis as a manifestation of chronic lung rejection?

To the Editor:

Idiopathic pleuroparenchymal fibroelastosis is a peculiar pulmonary fibrosis proposed by FRANKEL *et al.* [1] in 2003 and is almost the same concept as idiopathic pulmonary upper lobe fibrosis proposed by AMITANI *et al.* [2]. There are no known causes for fibrosis in idiopathic pleuroparenchymal fibroelastosis. Sometimes, pleuroparenchymal fibroelastosis (PPFE) has underlying diseases or conditions, such as collagen vascular diseases, anti-cancer chemotherapy, irradiation, asbestos exposure and bone-marrow transplantation [3]. Herein, we report the case of a female who received living-donor lung transplantation and died of pulmonary fibrosis, which was pathologically compatible with PPFE in addition to constrictive bronchiolitis, which is a manifestation of chronic lung allograft dysfunction (CLAD) [4].

A 30-yr-old female suffering from idiopathic pulmonary arterial hypertension underwent living-donor lung transplantation surgery and received a right lower lobe from her younger sister and a left lower lobe from her mother in December 2003. 20 months after the lung transplantation she had dyspnoea and a chest radiograph disclosed bilateral ground-glass shadows. 1 month later, right open lung biopsy was performed and a diagnosis of interstitial pneumonia was obtained. Pulse therapy with methylprednisolone slightly improved her condition and prednisolone was administered after the pulse therapy. However, bilateral interstitial opacities gradually deteriorated (fig. 1) with increased dyspnoea. 49 months after the lung transplantation, her daily life had worsened to almost whole-day bed rest. 18 days prior to her death she noticed fever and general fatigue and was admitted to our hospital (Dept of Respiratory Medicine, Fukuoka University Hospital, Fukuoka, Japan). Pulse therapy using methylprednisolone and antimicrobial and antifungal drugs were administered, without effect. She died 52 months after the lung transplantation. Histological specimens (figs 1a and b) were obtained at autopsy and chest computed tomography (fig. 1c) was obtained 51 months after lung transplantation (1 month before her death). The autopsy revealed a fibrously thickened visceral pleura and marked deposition of elastin just beneath the thickened pleura in both lungs. Alveoli filled with collagen (intra-alveolar fibrosis) were found around the border between the subpleural elastosis and the less involved lung parenchyma. There were foci of constrictive bronchiolitis surrounded by intra-alveolar fibrosis in the lung parenchyma, away from the pleural/subpleural fibrosis and elastosis. The pleural fibrosis, subpleural elastosis and intra-alveolar fibrosis observed in the present case were identical to the pathological features of PPFE described by FRANKEL *et al.* [1] and were considered as pulmonary fibrosis secondary to lung transplantation.

In 2003, KONEN *et al.* [5] reported fibrosis of the upper lobes in seven lung transplant recipients. In 2005, PAKHALE *et al.* [6] also

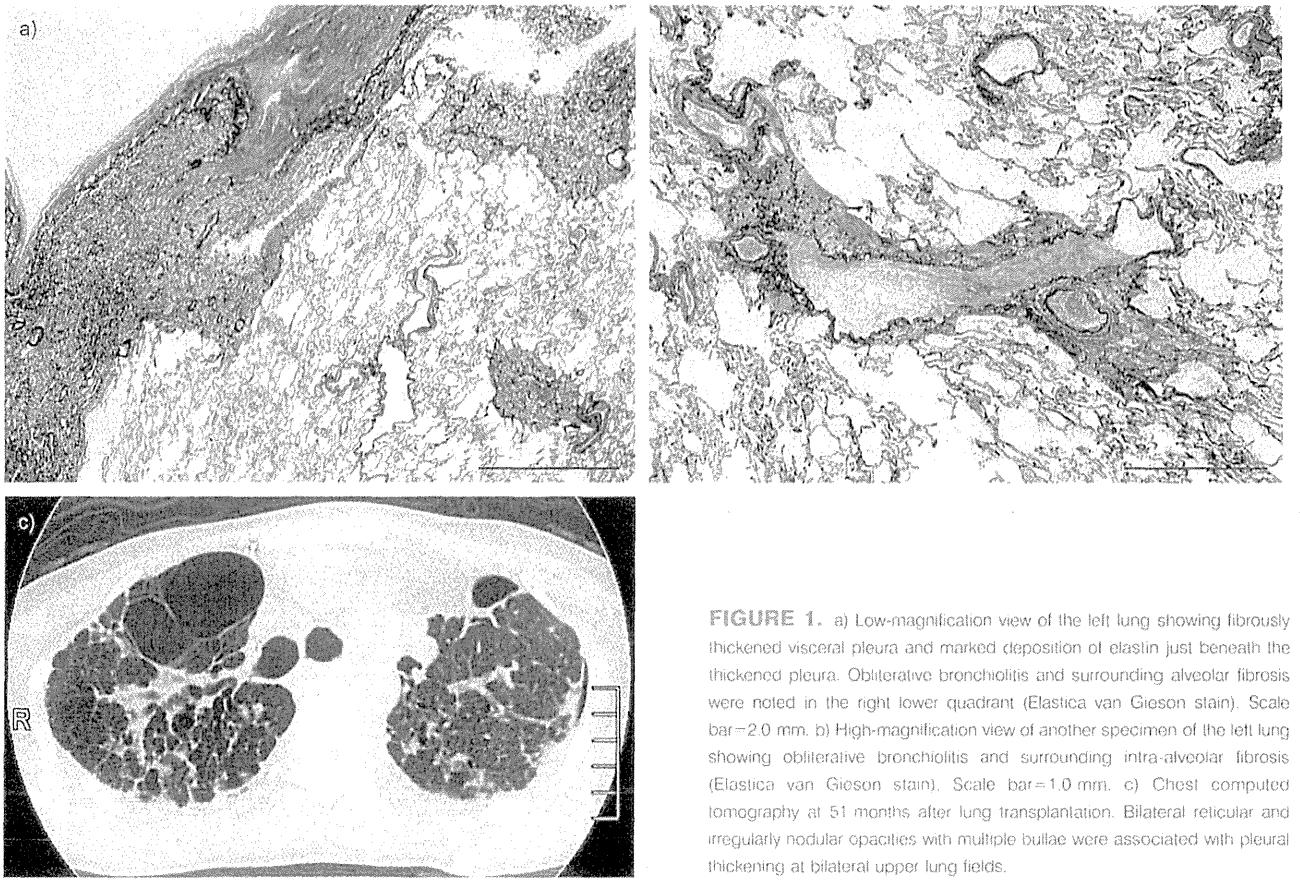

FIGURE 1. a) Low-magnification view of the left lung showing fibrously thickened visceral pleura and marked deposition of elastin just beneath the thickened pleura. Obliterative bronchiolitis and surrounding alveolar fibrosis were noted in the right lower quadrant (Elastica van Gieson stain). Scale bar=2.0 mm. b) High-magnification view of another specimen of the left lung showing obliterative bronchiolitis and surrounding intra-alveolar fibrosis (Elastica van Gieson stain). Scale bar=1.0 mm. c) Chest computed tomography at 51 months after lung transplantation. Bilateral reticular and irregularly nodular opacities with multiple bullae were associated with pleural thickening at bilateral upper lung fields.

reported the same concept of the disease in 13 lung transplant recipients. The authors presented imaging characteristics of the fibrosis that started in the upper lobe and expanded to other lobes. Some patients were autopsied, but they did not exhibit any specific histological features for the fibrosis, although some patients had co-existent constrictive bronchiolitis. The authors also suggested the clinical co-existence of bronchiolits obliterans syndrome (BOS) and upper lobe fibrosis in the analysis of the results of respiratory function tests. They concluded that the fibrosis was a novel presentation of chronic allograft dysfunction in lung transplant recipients.

Using autopsy materials, we examined the histological features of the fibrosis in a lung transplant recipient and found that the histology of the fibrosis was identical to that of PPFE. We also identified constrictive bronchiolitis surrounded by peribronchiolar intra-alveolar fibrosis. Intra-alveolar fibrosis and subpleural elastosis are fundamental features of PPFE [7]. These findings raise the possibility that a close relationship exists between upper lobe fibrosis and constrictive bronchiolitis and that the organising process involving the peripheral airway and alveoli without resolution could be the initial step for the disorder, with peripheral airway-dominant lesions resulting in BOS and alveolus-dominant lesions resulting in pulmonary fibrosis.

According to the revised consensus classification of lung allograft rejection by the International Society for Heart and Lung Transplantation (ISHLT) [8], upper lobe fibrosis, as described by KONEN *et al.* [5] and PAKHALE *et al.* [6], is a late-onset complication after lung transplantation that is considered an unhelpful observation because of the lack of specificity and the difficulty in the interpretation of transbronchial biopsy specimens. However, in 2011, SATO *et al.* [9] proposed the concept of restrictive allograft dysfunction (RAS) as a form of CLAD. CLAD was defined as an irreversible decline in forced expiratory volume in 1 s (FEV_1) to <80% of the baseline, and RAS was defined as CLAD with an irreversible decline in total lung capacity (TLC) to <90% of the baseline. As FEV_1 and TLC in our patient fell to 30% and 44%, respectively, of the best levels after lung transplantation (baselines) (fig. 2), her ventilatory impairment met the criteria of RAS.

Because RAS was defined functionally, its histological characteristics have not been well established. SATO *et al.* [9] characterised RAS as various stages of diffuse alveolar damage and extensive fibrosis in the alveolar interstitium with or without constrictive bronchiolitis lesions. In this report, we presented PPFE as a possible pathological phenotype of RAS. Our case might share pathological characteristics with upper lobe fibrosis, which were described by KONEN *et al.* [5] and PAKHALE *et al.* [6].

The close relationship between constrictive bronchiolitis and intra-alveolar fibrosis in the present case suggests that PPFE and constrictive bronchiolitis share a common pathway to

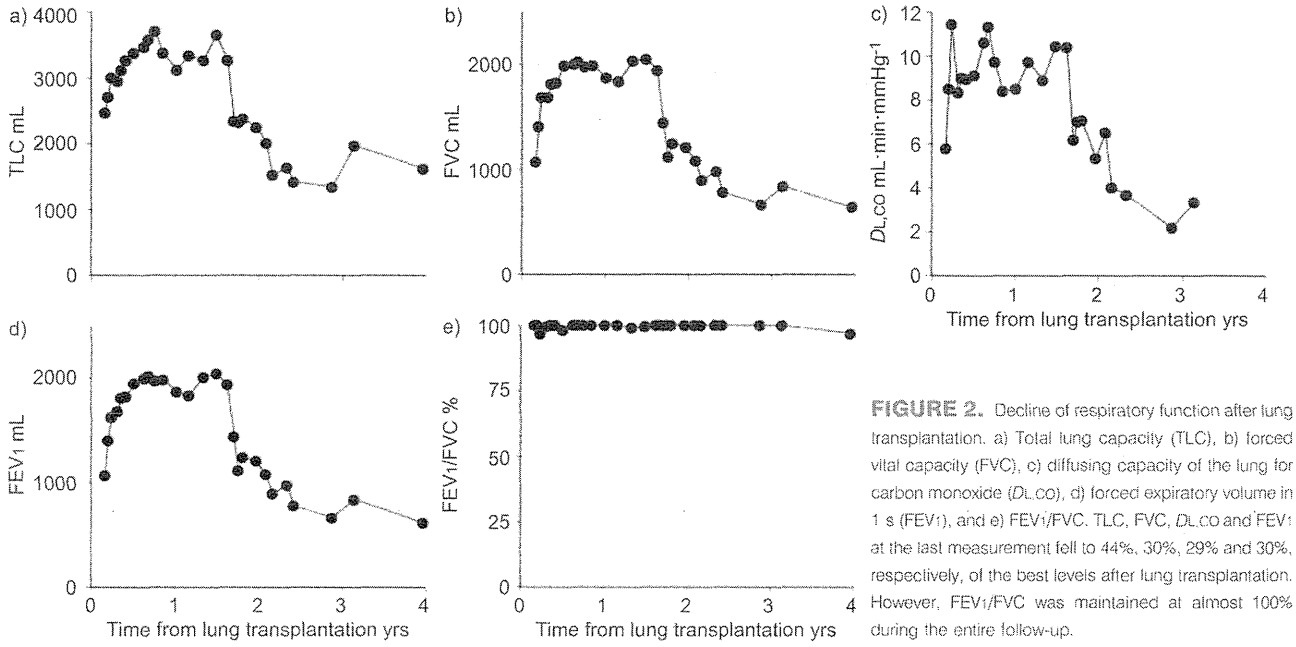

FIGURE 2. Decline of respiratory function after lung transplantation. a) Total lung capacity (TLC), b) forced vital capacity (FVC), c) diffusing capacity of the lung for carbon monoxide (*D*L,CO), d) forced expiratory volume in 1 s (FEV_1), and e) FEV_1/FVC. TLC, FVC, *D*L,CO and FEV_1 at the last measurement fell to 44%, 30%, 29% and 30%, respectively, of the best levels after lung transplantation. However, FEV_1/FVC was maintained at almost 100% during the entire follow-up.

CLAD. PPFE may be a possible pathological phenotype of RAS and a manifestation of CLAD, as is constrictive bronchiolitis.

We showed that lung transplantation is another underlying condition that could induce secondary PPFE. Further studies are needed to elucidate the pathophysiology of transplantation-associated PPFE, which is sometimes a lung manifestation of the chronic graft-*versus*-host disease observed in bone-marrow transplantation, but is sometimes pathology of CLAD in lung transplantation.

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Support Statement: This work was partly supported by a grant to the Diffuse Lung Diseases Research Group from the Ministry of Health, Labour and Welfare, Japan.

Statement of Interest: None declared.

REFERENCES

1 Frankel SK, Cool CD, Lynch DA, *et al.* Idiopathic pleuroparenchymal fibroelastosis: description of a novel clinicopathological entity. *Chest* 2003; 126: 2007–2013.
2 Amitani R, Niimi A, Kuse F. [Idiopathic pulmonary upper lobe fibrosis]. *Kokyu* 1992; 11: 693–699.
3 von der Thusen JH, Hansell DM, Veys PA, *et al.* Pleuroparenchymal fibroelastosis in patients with pulmonary disease secondary to bone marrow transplantation. *Modern Pathol* 2011; 24: 1633–1639.
4 Woodrow JP, Shlobin OA, Barnett SD, *et al.* Comparison of bronchiolitis obliterans syndrome to other forms of chronic lung allograft dysfunction after lung transplantation. *J Heart Lung Transplant* 2010; 29: 1159–1164.
5 Konen E, Weisbrod GL, Pakhale S, *et al.* Fibrosis of the upper lobes: a newly identified late-onset complication after lung transplantation? *AJR Am J Roentogenol* 2003; 181: 1539–1543.
6 Pakhale SS, Hadjiliadis D, Howell DN, *et al.* Upper lobe fibrosis: a novel manifestation of chronic allograft dysfunction in lung transplantation. *J Heart Lung Transplant* 2005; 24: 1260–1268.
7 Reddy TL, Tominaga M, Hansell DM, *et al.* Pleuroparenchymal fibroelastosis: a spectrum of histopathological and imaging phenotypes. *Eur Respir J* 2012; 40: 377–385.
8 Stewart S, Fishbein MC, Snell GI, *et al.* Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. *J Heart Lung Transplant* 2007; 26: 1229–1242.
9 Sato M, Waddell TK, Wagnetz U, *et al.* Restrictive allograft syndrome (RAS): a novel forms of chronic lung allograft dysfunction. *J Heart Lung Transplant* 2011; 30: 735–742.

DOI: 10.1183/09031936.00103912

Current Respiratory Medicine Reviews, 2013, *9*, 229-237

Pleuroparenchymal Fibroelastosis: Its Clinical Characteristics

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Abstract: Pleuroparenchymal fibroelastosis (PPFE) is a rare pulmonary fibrosis that is clinically characterized by upper-lobe predominant fibrosis. PPFE is a slowly progressive disorder and its first symptom is dyspnea or dry cough. Chest pain because of pneumothorax may be the first symptom in some patients. Patients with PPFE are slender with a flat rib cage or abnormally narrowed anterior–posterior thoracic dimension. Decreases in forced vital capacity, total lung capacity, and diffusing capacity are respiratory-function characteristics of PPFE, similar to those seen in idiopathic pulmonary fibrosis (IPF). The most remarkable difference in clinical features between PPFE and IPF is imaging findings, with upper-lobe-predominant lesions in PPFE and lower-lobe-predominant lesions in IPF.

Keywords: Pleuroparenchymal fibroelastosis (PPFE), pulmonary upper lobe fibrosis, pulmonary fibrosis (IPF).

INTRODUCTION

Pleuroparenchymal fibroelastosis (PPFE) was first reported by Frankel *et al.* [1]. PPFE can occur without any etiology or underlying diseases (idiopathic PPFE), or with underlying diseases or conditions. Idiopathic PPFE has been listed as one of the rare idiopathic interstitial pneumonias (IIPs) in the revised international multidisciplinary classification of the IIPs [2]. One of its characteristics is upper-lobe-dominant progressive pulmonary fibrosis with a peculiar histopathology consisting of visceral pleural thickening with collagenous fibrosis, subpleural elastosis, and intraalveolar collagenous fibrosis [1].

The clinical course of PPFE progresses slowly and is similar to that of chronic fibrosing interstitial pneumonias, such as idiopathic pulmonary fibrosis (IPF) and fibrotic nonspecific interstitial pneumonia (NSIP). Although the fibrosis is rarely encountered in clinical practice, since the appearance of the paper by Frankel *et al.* [1], an increasing number of studies have examined PPFE. In this chapter, the clinical characteristics of PPFE will be discussed, together with the historical changes in the concept of pulmonary upper-lobe fibrosis.

PULMONARY UPPER-LOBE FIBROSIS: HISTORY OF THE CONCEPT

There is a long history of pulmonary upper-lobe fibrosis with unknown etiology. In 1975, Davies *et al.* reported five patients with idiopathic progressive pulmonary fibrosis [3]. The fibrosis was confined to the upper parts of the lung and resembled pulmonary lesions in ankylosing spondylitis [4]. Their clinicopathological features, such as progressive dyspnea, marked weight loss, severe restrictive impairment, and upper-lobe-dominant fibrosis, are similar to those observed in PPFE today. Similar cases had already been published in 1962 and 1966 as "chronic idiopathic pneumonia" [5] and "idiopathic cavitation of the lung" [6]. Later, similar cases of upper-lobe fibrosis were reported under the terms "pulmonary upper-lobe fibrocystic changes" [7], "pulmonary apical fibrocystic disease" [8], or "idiopathic progressive pleuropulmonary fibrosis" [9]. The common radiographic feature in these papers was upper-lobe fibrosis and not lower-lobe fibrosis, as seen in IPF.

In 1992, Amitani *et al.* reported 13 patients with upper-lobe-localized pulmonary fibrosis with unknown etiology [10]. This report, written in Japanese, included nine cases with pathological analysis (open lung biopsy, three cases; autopsy, two cases; transbronchial lung biopsy, four cases), which represented the largest number of patients at that time. In that paper, the authors named the disorder idiopathic pulmonary upper-lobe fibrosis (idiopathic PULF) and presented its clinical and histological characteristics as follows: 1) slender stature with flattened thoracic cage; 2) progressive bilateral subpleural fibrosis in the upper lobes with bullae but without honeycombing; 3) recurrent pneumothorax; 4) no extrathoracic lesions; 5) absence of acid-fast bacilli and lack of effect of antituberculous drugs; 6) aspergillus infection may be superimposed; and 7) slowly progressive and fatal condition with 10–20 years of presentation. The paper by Amitani *et al.* was followed by several Japanese case reports describing idiopathic PULF [11-16], before the paper on PPFE by Frankel *et al.* [1] appeared in 2004.

A Japanese review article by Kawabata *et al.* [17] on the histopathological characteristics of idiopathic PULF was published in 2003, when the fibrosis was well known among the interstitial lung disease community of Japanese pulmonologists, and idiopathic PULF was also called "Amitani disease", in his honor. Kawabata described the pathological features as follows: subpleural atelectatic induration with the proliferation of elastic fibers and intraluminal organization or intraalveolar fibrosis in the upper lobes, and fibrously thickened pleura. Such pathological features are totally different from those of IPF.

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1875-6387/13 $58.00+.00

A year after the publication of the paper by Kawabata *et al.*, Frankel *et al.* reported five patients with upper-lobe-dominant pulmonary fibrosis characterized by intense fibrosis of the visceral pleura and subpleural fibrosis with a mixture of elastic tissue and dense collagen. They termed the fibrosis pleuroparenchymal fibroelastosis [1].

"Subpleural atelectatic induration with the proliferation of elastic fibers and intraluminal organization or intraalveolar fibrosis" described by Kawabata *et al.* [17] is histologically identical to "subpleural fibrosis with a mixture of elastic tissue and dense collagen" reported by Frankel *et al.* [1]. The main histological features are almost identical in these two fibroses.

Subsequently, many papers on PULF continued to be published in Japan [18-26]. Recently, however, an increasing number of reports have appeared in English on the fibrosis named PPFE [27-33], and PPFE is now globally accepted as a representative nomenclature for this disorder (Table **1**). However, PPFE is a diagnostic term that expresses histological features. The term "upper-lobe fibrosis" is necessary for clinicians, especially when discriminating the disorder from IPF in clinical practice. Therefore, in my opinion, PULF is to be used for a clinical or clinical-radiological-pathological diagnosis, and PPFE is to be used for a histological pattern, as shown in the diagnosis of IIPs in the international multidisciplinary consensus classification of the IIPs [34]. At present, however, surgical lung biopsy is indispensible for the definite diagnosis of this rare pulmonary fibrosis.

Table 1. Same or Similar Disease Concepts as Pleuroparenchymal Fibroelastosis

Chronic idiopathic pneumonia [5] 1962
Idiopathic cavitation of lung [6] 1966
Idiopathic progressive pulmonary fibrosis [3] 1975
Pulmonary upper lobe fibrocystic changes [7] 1978
Upper lobe fibrosis and cavitation [44] 1980
pulmonary apical fibrocystic disease [8] 1981
idiopathic progressive pleuropulmonary fibrosis [9] 1984
Apical pulmonary fibrosis [37] 1988
Idiopathic pulmonary upper lobe fibrosis [10] 1992
Marked pulmonary fibrosis in the upper lung field [12] 1999
Marked pulmonary fibrosis in the upper lobe [13] 1999
Upper lobe fibrosis [36, 41] 2003, 2005
Upper lobe-dominat pulmonary fibrosis [20] 2010

There is a controversy regarding the extent of the fibrosis: whether the lesions are confined to upper lobes ("pure" PULF or so-called Amitani disease), extend to adjacent lobes, or present as isolated usual interstitial pneumonia (UIP)-like lesions in the lower lobes in addition to the upper-lobe fibrosis [23]. Some pulmonologists in Japan claim that pulmonary "upper-lobe-localized" fibrosis (Amitani disease) should be discriminated from pulmonary "upper-lobe-dominant" fibrosis, because they think that the latter might be a variant of UIP or an upper-lobe manifestation of other fibrosing lung diseases, and Amitani disease is a peculiar form of fibrosis that is confined to the upper lobes, unlike what is observed in other IIPs.

Although the name PULF was originally given to pulmonary fibrosis localized only in the upper lobes, and not extending to other lobes [10, 17], it has been disclosed that the number of patients with fibrosis involving not only the upper lobes, but also other lobes, is much greater than that of patients with upper-lobe-localized fibrosis. Moreover, the boundary between upper-lobe-localized fibrosis and upper-lobe-dominant fibrosis has become ambiguous in clinical practice. In addition, it has also been disclosed that the PPFE pattern as a histological finding is associated with many of the diseases described below; thus, it appears more important today to differentiate idiopathic PPFE from secondary PPFE or PPFE with underlying diseases, rather than to differentiate upper-lobe-localized fibrosis from upper-lobe-dominant fibrosis.

CLINICAL CHARACTERISTICS

In this section, PULF is considered to be the same as PPFE as a clinical entity. Therefore, henceforth, I will use the term PPFE as being representative of both types of pulmonary fibrosis, PPFE and PULF, either upper-lobe-localized or upper-lobe-dominant fibrosis. Moreover, we have to differentiate the use of the term PPFE as a clinical disease from the PPFE histological pattern seen in various diseases.

Normally, clinical characteristics should be separately discussed in idiopathic and secondary forms of PPFE. As seen in patients with ankylosing spondylitis [4], a considerable number of patients with PPFE have underlying diseases or conditions that might be relevant to its occurrence and development (Table **2**). In this chapter, the term PPFE will be used for both fibrosis with and without underlying diseases, otherwise specified as idiopathic PPFE or secondary PPFE (or PPFE with underlying diseases), respectively.

Table 2. Underlying Diseases or Conditions that may be Associated with Pleuroparenchymal Fibroelastosis (PPFE)

Idiopathic PPFE
Radiation
Anticancer chemotherapy
Bone marrow- or stem cell-transplantation
Lung transplantation
Occupational dust exposure
Asbestos
Aluminum
Infection
Aspergillus
Mycobacterium avium-intracellulare
Hereditary PPFE ~ PPFE with family history
Autoimmune diseases
Rheumatoid arthritis
Ulcerative colitis
Psoriasis
Ankylosing spondylitis
Hypersensitivity pneumonitis