## Abstracts of Outstanding Presentation (2)

# Significance of Angiogenesis in the Intra-alveolar Fibrosis in Interstitial Pneumonia

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### Introduction

Intra-alveolar fibrosis is known to cause structural remodeling in interstitial pneumonia (IP). In this study, we examined the tendency of intra-alveolar fibrosis formation and the degree of angiogenesis in intra-alveolar fibrosis in usual interstitial pneumonia (UIP), nonspecific interstitial pneumonia (NSIP), and diffuse alveolar damage (DAD). Moreover, using immnohistochemistry and real time-polymerase chain reaction (RT-PCR), we examined the expression of vascular endothelial growth factor A (VEGF-A) and its receptor-2 (VEGF-R2), which are known to be essential for angiogenesis, and discussed the significance of angiogenesis in IP.

#### Materials and Methods

By histological examination, we counted bud-type, mural incorporation-type and obliterative-type intra-alveolar fibrosis in each case of IP (NSIP: n=24; UIP: n=18; and DAD: n=16). Then, we classified the degree of angiogenesis (number of occurrences of intra-alveolar fibrosis with angiogenesis/number of occurrences of intra-alveolar fibrosis) in each case (>50%: +++; 25%-50%: ++; 1%-25%: +; and 0%: -) and compared the degree of angiogenesis and the type of intra-alveolar fibrosis. For immunohistochemical experiments, we performed immunostaining on paraffin-embedded sections using a mouse monoclonal antibody and a rabbit polyclonal antibody for VEGF-A and VEGF-R2, respectively. In addition, we performed RT-PCR using TaqMan probes for VEGF-A and VEGF-R2.

#### Results

In NSIP, there were many occurrences of mural incorporation-type intra-alveolar fibrosis and some bud-type intra-alveolar fibrosis, and angiogenesis, as determined by positive reactivity for CD34, was observed in both types (Fig. 1A and B). In UIP, mostly mural incorporation-type intra-alveolar fibrosis was seen, along with a small amount of angiogenesis (Fig. 1C). In DAD, there was mostly obliterative-type and some bud-type intra-alveolar fibrosis, and angiogenesis was rare (Fig. 1D). Overall, more angiogenesis in intra-alveolar fibrosis was

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Fig. 1 Histological features of angiogenesis in intra-alveolar fibroses. (A) Bud-type intra-alveolar fibrosis in NSIP showing blood vessels (arrow). (B) Mural incorporation-type intra-alveolar fibrosis in NSIP showing blood vessels (arrows). (C) Mural incorporation-type intra-alveolar fibrosis in UIP showing no blood vessels. (D) Obliterative-type intra-alveolar fibrosis in DAD showing no blood vessels.



Fig. 2 Angiogenesis in cases of IP. Angiogenesis is common in NSIP, less apparent in UIP, and rare in DAD.



Fig. 3 Angiogenesis in each type of intra-alveolar fibrosis. Bud-type intra-alveolar fibrosis has more angiogenesis than do the other two types in NSIP. In DAD, angiogenesis is only occasionally found in bud-type intra-alveolar fibrosis.

seen in NSIP (**Fig. 2**); among the three types of intra-alveolar fibrosis, the bud type had the highest degree of angiogenesis (**Fig. 3**). The level of VEGF-A mRNA expression was significantly higher in NSIP than in UIP or DAD.

## Discussion

Angiogenesis is usually observed in wound healing. Here, we show that angiogenesis is frequently observed in NSIP and is much less common in intra-alveolar fibrosis in UIP and DAD. VEGF-A plays a role in the angiogenesis observed in NSIP and is known to increase the expression of some of matrix metalloproteinases (MMPs). In organizing pneumonia and NSIP, high levels of MMP-2 have been reported. We suppose that angiogenesis in fibrotic lesions of NSIP may correlate with the absorption of extracellular matrices, which leads to wound healing, and that the scarcity of angiogenesis in fibrotic lesions of UIP and DAD may be a factor indicating a poor prognosis in these types of IP.