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LETTER TO THE EDITOR

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Vasohibin-1 and miR-720 expression in diffuse pulmonary capillary hemangiomatosis-like changes associated with pulmonary hypoplasia

To the Editor

Pulmonary capillary hemangiomatosis (PCH), first described by Wagenvoort *et al.* in 1978, is an extremely rare angioproliferative disease. PCH is histopathologically characterized by extensive capillary proliferation mainly in alveolar septum, partly also in blood vessels, bronchioles or other neighboring structures. PCH-like changes may be observed following other abnormal conditions such as pulmonary veno-occlusive disease (PVOD)[1] and therefore are considered to be reactive changes but their etiology is unknown. Vasohibin-1 (VASH-1) is an endothelium-derived negative feedback regulator of angiogenesis,[2] upregulated by vascular endothelial growth factor (VEGF) or fibroblast growth factor, and is negatively regulated by miRNA such as miR-720.[3] The miR-720 - VASH-1 pathway is reported to be significantly involved in the process of vascular repair in coronary artery diseases, but its status has not been studied in PCH. In this study, we report the clinical and pathological characteristics and status of the miR-720-VASH-1 pathway activation of a patient, who developed PCH-like changes in the course of pulmonary hypertension due to pulmonary hypoplasia.

The 16-year-old female patient, who was diagnosed as neonatal asphyxia and meconium aspiration syndrome at birth, was transferred to our hospital. She was born full-term without apparent congenital anomalies. After birth, she further presented refractory respiratory failure and recurrent upper respiratory tract infection without development of respiratory distress syndrome. She was diagnosed as pulmonary hypoplasia and pulmonary hypertension (Group 3.5 based on the classification of *paediatric pulmonary arterial hypertension in the 6th World Symposium on Pulmonary Hypertension*[4]) at 5 months old by pulmonary perfusion imbalance on scintigraphy and elevated pulmonary artery pressure on cardiac catheterization. She had been on continuous oxygen therapy and unable to walk outside. Respiratory failure, hypoxia, exertional dyspnea and pulmonary hypertension gradually progressed, leading to severe right heart failure. Sildenafil administration was started at this time, nevertheless, respiratory failure and pulmonary hypertension gradually progressed further. She was transferred to our hospital for lung transplantation at the age of 16. Oxygen saturation (SpO_2) at this point was 60% with 10 L/min O_2 by mask, and demonstrated New York Heart Association Class IV. A plain chest X-ray and computed tomography scan revealed a significant enlargement of the mediastinal shadow, dilatation of bilateral pulmonary arteries (46 mm in diameter at the main pulmonary trunk), diffuse ground glass opacities and thickening of interlobular septa. Mean pulmonary arterial pressure was 69 mmHg (normal range <20 mmHg). Living-donor bilateral lung transplantation was performed, accordingly.

Macroscopically, both lungs were approx. 70% smaller than the normal range of those of the corresponding age (left 13 cm, light 15 cm in longitudinal axis). Microscopically, alveolar septa were irregularly thickened with extensive capillary proliferation forming double or more layers and alveoli were variable in size and shape (Fig. 1a,b). The capillary proliferation partly expanded into the walls of bronchioles and blood vessels (Fig. 1b). These PCH-like changes were observed almost homogenously and diffusely over 70–80% of the alveoli areas in all lobes. Capillaries are mostly small in size, lined by flat endothelial cells without remarkable reactive changes. No microthrombi or capillaritis were observed. There were a few hemosiderin-laden macrophages in alveolar spaces. Interlobular space was expanded due to severe edema with lymphatic dilatation (Fig. 1a). No apparent occlusion of veins or venules was detected (Fig. 1a). Mild medial thickening in small to middle-sized pulmonary arteries, so-called muscularization, without marked arterial deformity, for example, plexiform vascular changes, was observed. The radial alveolar count of this patient was on average six (normal range 8–12 in teenagers), suggesting immaturity of the lungs. Finally, PCH-like feature and hypoplasia of the lungs were histopathologically confirmed.

Microvessel density (MVD) and expression of VASH-1 and miR-720 were evaluated in the patients' lung specimens, as well as nonpathological lung tissues resected due to

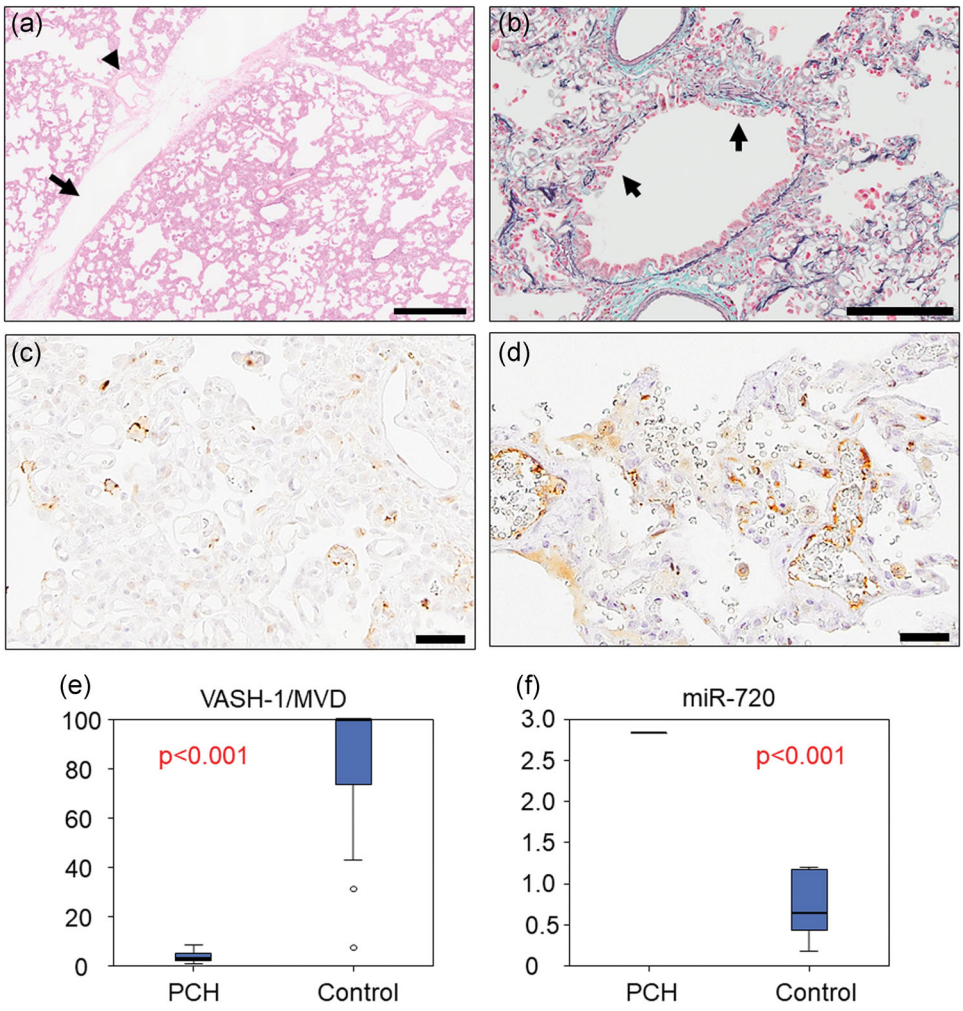

Figure 1 The histopathological images of pulmonary capillary hemangiomatosis (PCH)-like changes (**a**) Hematoxylin and eosin stain. Alveolar septum was thickened and alveoli distrbuted irregulary in size and shape. Interlobular space was expanded due to marked edema (arrow). No apparent occlusion was detected in veins or venules (arrow head). Scale bar: 1 mm. (**b**) Elastica-Masson stain. Alveolar septum with extensive proliferation of the capillaries showing double or more layers. The capillary proliferation partly involving walls of bronchioles (arrow). Scale bar: 200 μm. (**c**,**d**) Immunoreactivity of VASH-1 in PCH-like change (**c**) and normal lung (**d**). VASH-1 immunoreactivity was focally detected in endothelial cells of the capillaries in the PCH-like change, while diffusely present in normal lung. Scale bar: 50 μm. (**e**) VASH-1/MVD was significantly lower in the PCH-like change than in normal lung ($P < 0.001$). (**f**) miR-720 expression was significantly higher in the PCH-like change than in normal lung ($P < 0.001$).

pneumothorax as a control ($n = 7$, mean age 18). Immunohistochemical staining was performed employing previously reported methods using antibodies shown in Table S1, and double immunostaining of Ki-67 identified by 3.3′-diaminobenzidine and CD31 by Vector-blue was performed.[5] The immunohistochemical evaluation was performed as previously reported.[5] Quantitative real-time polymerase chain reaction (PCR) was performed according to the manufacturer's recommendation (QIAGEN, Hilden, Germany). miR-720 expression was calculated as relative to U6 using miScript Primer Assays and miScript PCR System (QIAGEN) and ABI 7500 Fast System SDS Software (Applied Biosystems, CA, USA). VASH-1/MVD evaluated in the patients' lungs was significantly lower than that in normal lung tissues ($P < 0.001$, Wilcoxon-Mann-Whitney U) (Fig. 1c–e). In contrast, MVD, Ki-67/MVD and miR-720 expression in the patients' tissue was significantly higher than that in normal lung tissues (MVD, $P < 0.001$; Ki-67/MVD, $P < 0.001$; miR-720, $P < 0.001$, Wilcoxon-Mann-Whitney U) (Fig. 1f). The study protocol was approved by the Ethics Committee at the Tohoku University School of Medicine.

We reported a patient who presented diffuse PCH-like changes in both of her lungs that were developed in the course of pulmonary hypertension associated with pulmonary hypoplasia. In the tissues, miR-720 was significantly overexpressed and associated with abnormal VASH-1 inhibition. miR-720 is upregulated by extracellular signal-regulated kinase (ERK) signaling. ERK signaling activation

(e.g., Caveolin-1, Platelet-derived growth factor) has been reported in PCH. ERK signaling pathway is activated by VEGF, which also upregulates VASH-1.[2] Therefore, VEGF could exert dichotomic effects on VASH-1, the one promotes its activation through VEGF receptor 2[2] and others could inhibit through ERK signaling-miR-720 pathway. Results of previous studies demonstrated that VEGF could cause microvessel proliferation in PCH, suggesting that the latter inhibitory effects could act more dominantly than the former promoting effects. Based on our results, it is postulated that miR-720-VASH-1 pathway could play important roles in etiology of the PCH-like changes.

Some investigators have postulated that the PCH-like changes subsequently occur in other abnormal conditions such as PVOD.[1] We hypothesize that pulmonary hypoplasia of this particular patient might have led to severe pulmonary hypertension due to insufficient vascular bed and relative blood overflow,[4] that could have induced capillary proliferation as a form of compensatory gas exchange in the alveoli as well as reduction of vascular pressure. To the best of our knowledge, this is the first report of a patient, who developed PCH-like changes in the long course of pulmonary hypoplasia. We revealed that miR-720-VASH-1 pathway is activated in the lung tissues obtained from the patient.

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DISCLOSURE STATEMENT

None declared.

AUTHOR CONTRIBUTIONS

All the authors have contributed significantly to the content of the manuscript. RS and AK designed the study; RS performed the experiments in cooperation with YM and SH and prepared the manuscript; YK, CI, FF, ST and MW contributed to the pathological diagnosis; JT, ST, HN and YO provided clinical information; YS provided VASH-1 antibody; HS supervised the study; RS, AK and HS edited the manuscript. All the authors read and approved the final manuscript.

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