Functional microangiography of *in vivo* mouse pulmonary circulation

The underlying mechanisms responsible for the pathogenesis of pulmonary arterial hypertension (PAH) still remain to be fully elucidated. Structural and functional changes in pulmonary microvessels are critical for modulating pulmonary blood flow, which is essential for optimizing ventilation-perfusion matching. Ultimately, dysfunction of the pulmonary microvasculature plays a pivotal role in the pathogenesis of several serious lung diseases. Recently, the ability to produce targeted gene mutations in mice has provided a powerful tool for studying molecular mechanisms of PAH *in vivo*. However, the pulmonary vascular function is still not fully elucidated in the mouse model. One of the limiting factors for measuring pulmonary dynamics in this animal is its small size.

The visualization of the microvascular bed provides invaluable insight for evaluating vasomotor function and, furthermore, understanding the underlying mechanisms that trigger early vessel disorders. Our group designed a unique laboratory X-ray TV system to visualize 100- to 500-μm-diameter pulmonary vessels *in vivo* [1]. This conventional X-ray system contributed to an assessment of the intravital vascular network. However, the limited spatial and temporal resolution of this system necessitated a thoracotomy to expose the lung out of the thorax and made it difficult to assess pulmonary blood flow distribution within the intact chest of a small animal model. In the last decade the technological advances in angiography by synchrotron radiation (SR) microangiography have provided the temporal and spatial resolutions required to visualize microvessels of various organs both *ex vivo* and *in vivo*. In our previous study in SPring-8, we demonstrated the visualization of pulmonary microvessels in a closed-chest rat model and investigated functional changes of the microvessels in experimental pulmonary hypertension [2]. Through successive studies using a rat model, we endeavored to advance the unique SR microangiography technique for visualizing the pulmonary hemodynamics in the mouse model *in vivo* [3].

All animal experiments were performed at beamline **BL28B2**. Briefly, healthy adult C57BL/6 mice (~25 g body weight) were anesthetized and lungs were mechanically ventilated with a small rodent ventilator. Surgical preparation was performed as previously reported using a rat model [2], although major modifications of experimental settings were required for mice imaging. Specifically, right ventricle catheterization via the right jugular vein was performed with a hand-made catheter (outer diameter 0.7 mm) and iodinated contrast medium was directly injected into the right ventricle using a high-pressure injection pump at a speed of 10 ml/min.

SR microangiography was effective for visualizing dynamic changes in the pulmonary vessel caliber using iodinated contrast agent. We observed, frame by frame, that thecontrast agent circulated from the right ventricle to pulmonary vessels in real time. Figure 1 illustrates typical microangiographic images showing the middle portion of left and right lungs from an anesthetized mouse. In our experimental setting, the mouse, which has small thoracic size, was suitable

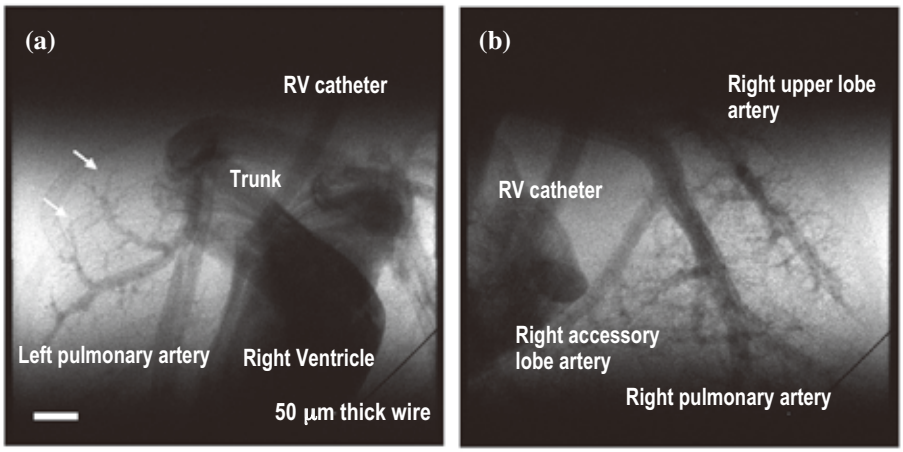

Fig. 1. Typical imaging pattern of mouse left **(a)** and right **(b)** lung circulation. A 50-μm-thick wire for calibration is depicted in the lower right area. **(a)** White arrows indicate the 3rd branches of the left middle lobe artery with less than 100 μm internal diameter. **(b)** Major arteries of the right lung (upper lobe artery, accessory lobe artery and right pulmonary artery) are all evident in a single region of interest. Scale bar = 1000 μm.

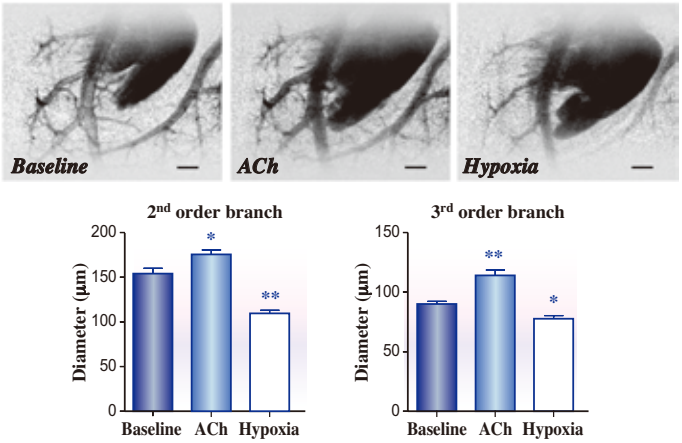

Fig. 2. **Top**: Typical microangiogram of *Baseline*, acetylcholine (*ACh*), and acute 10%-O_2 hypoxic exposure (*Hypoxia*). Smaller arteries partly disappeared in response to hypoxia. Scale bar = 1000 μm. **Bottom**: Magnitude of response to *ACh* and *Hypoxia* in each measurable branching generation (2nd, n=26, 3rd, n=15 vessels). Data show means +/– SE. *P<0.05, **P<0.01, significant difference from baseline.

for including the heart, lung, and most of the large to small pulmonary vessels within a single image. The diameter of the pulmonary trunk (~2 mm) and the 1st (~300 μm) to 3rd (~100 μm) branches from the left main axial artery could be measured, even when smaller than 100 μm. After the baseline angiogram was completed, the second and third angiograms were taken after the administration of a vasodilator (acetylcholine) and a vasoconstrictor (acute 10%-O_2 hypoxic exposure), respectively (Fig. 2). These stimuli changed the diameter of the 2nd and 3rd branches, which are pulmonary resistant vessels <~200 μm diameter (Fig. 2), but not the diameter of the left main axial artery and 1st branches. These analyses contribute to elucidating the regional differences in the vasomotor function among the series-arranged pulmonary vascular segments, and moreover, the target vascular site of the pathological dysfunction in lung diseases. We could also measure beat-by-beat dynamics of large conductance vessels by image analysis. The conductance vessel caliber oscillations in the left main axial artery were smaller and delayed relative to the trunk in the normal mouse (Fig. 3). In all mice, there were approximately 6 frames within each cardiac cycle. At the same time, we could estimate the pulmonary transit time of blood flow using cine imaging of single scan at a speed of 30 frames/s (1 scan = 100 frames over 3 s). The number of frames was counted between the time the contrast medium first appeared in the right ventricle and the time the contrast medium appeared in the left ventricle. Not surprisingly, the injected contrast agent quickly passed through gas-exchange vessels due to the high heart rate (over 400 beats per minute), and therefore, the calculated transit time was approximately within one second in the baseline (0.8 s). We could detect transit time prolongation (1.3 s, $p<0.05$) in acute hypoxic exposure. The transit time and conductance vessel fluctuation are determined by multiple cardiopulmonary factors (e.g., cardiac output, heart rate, and pulmonary vascular resistance and compliance); therefore, these parameters provide invaluable information concerning the cardiopulmonary hemodynamic status in cardiac and lung diseases.

In summary, we have exploited the full potential of monochromatic SR microangiography to show, for the first time, the ability to visualize pulmonary hemodynamics in a mouse model *in vivo*. SR microangiography is a powerful tool for assessing pulmonary hemodynamics in unprecedented detail in mice. Importantly, it can now provide us with the ability to assess the various neurohumoral pathways that modulate the pulmonary vasculature in specific gene-targeted knock-out and transgenic mice. Ultimately, future studies using SR microangiography on transgenic mice will provide important new insights into the pathophysiology of pulmonary dysfunctions and functional adaptation in cardiopulmonary disease.

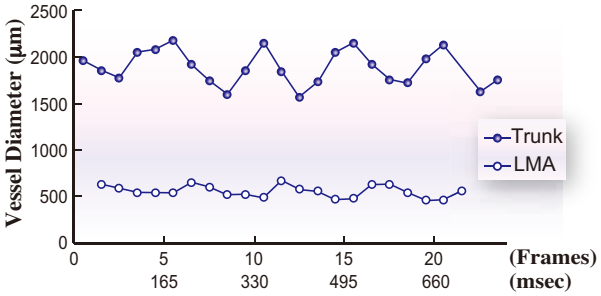

Fig. 3. Fluctuation of vessel diameters. The changes in internal diameter from sequential images of single scan (first 1 s of 3 s scan at 30 frame/s) were measured at the pulmonary trunk (Trunk) and left main axial artery (LMA). Each single frame had a shutter open time of 1.3 ms and a read-out time of approximately 33 ms.

Takashi Sonobe[a,*], Keiji Umetani[b] and Mikiyasu Shirai[a]

[a] Department of Cardiac Physiology, National Cerebral and Cardiovascular Center Research Institute
[b] SPring-8/JASRI

*Email: sonobe@ri.ncvc.go.jp

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