Shunt-type plexiform lesions identified in the Sugen5416/hypoxia rat model of pulmonary arterial hypertension using synchrotron-based phase-contrast micro-CT

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To the Editor:

We recently described four distinct types of plexiform lesions in human idiopathic and familial pulmonary arterial hypertension (PAH) [1], visualising the three-dimensional lesion structure using synchrotron-based phase-contrast micro-computed tomography (SPµCT). Two types, 1 and 2, are shunt-type lesions that connect pulmonary arteries to the bronchial circulation: type 1 to the vasa vasorum, and type 2 to peribronchial vessels. Type 3 lesions are found peripherally in the lung as spherical structures abruptly terminating the distal pulmonary artery/arteriole, and type 4 lesions are characterised by recanalisation of an occluded artery/arteriole. Our observation of type 1 and type 2 lesions in PAH supports previous work that demonstrated intrapulmonary bronchopulmonary anastomoses (IBAs) connected to plexiform lesions in human PAH, suggesting that shunting of blood can occur within lesions in the setting of supra-systemic pulmonary arterial pressure [2]. Further haemodynamic studies of distinct subtypes of plexiform lesions have been hampered by the lack of available animal models with plexiform lesions representative of the full range of lesion types found in human disease. Plexiform lesions have previously been described in the Sugen5416/hypoxia rat model of pulmonary hypertension when time until sacrifice following hypoxia is extended to 13–14 weeks. Initially plexiform lesions were identified within the pulmonary artery, as well as in the form of aneurysm-like lesions projecting outside the vessel lumen [3], and recently the latter type was shown to form in supernumerary arteries [4]. However, neither study observed plexiform lesions communicating with the bronchial circulation, possibly because of methodological limitations of the histological analysis.

Here, we set out to further characterise the range of plexiform lesion types in the prolonged Sugen5416/hypoxia rat model using SPµCT combined with injections of a radiopaque dye. As previously suggested by our group and others [5], SPµCT grants superior three-dimensional imaging of biological structures with low and homogenous attenuation and is an emerging tool in the fields of pulmonary vascular physiology and digital pathology.

The well-established Sugen5416/hypoxia model, combining vascular endothelial growth factor receptor 2 inhibitor injections with a second hit of hypoxia, was used as initially described [6], but sacrifice was delayed until 15 weeks post injections. No animals died during the study period. Following removal of the heart–lung block, the main pulmonary artery was injected with a radiopaque green dye (CDI's Tissue Marking Dye; Cancer Diagnostics, Durham, NC, USA), diluted with an equal volume of tap water to ensure low viscosity. Super-resolution x-ray radiographies of paraffin-embedded lobes were acquired using a laboratory setup as previously described [7], confirming the presence of contrast agent (radiopaque green dye) and thus enabling tracing of corresponding areas between individuals for subsequent high-resolution SPµCT imaging, which was performed at the X02DA TOMCAT beamline of the Swiss Light Source at the Paul Scherrer Institute, Switzerland [1, 5]. In short, 4× scan volumes (4.2×4.2×3.5 mm^3) with an effective voxel size of $(1.63\ \mu m)^3$ were acquired from selected areas of interest. The algorithm of Paganin *et al.* [8] and the gridrec algorithm [9] were applied for phase retrieval and tomographic reconstruction, respectively, using a Fiji plugin available at the beamline. Three-dimensional analysis was performed in Fiji [10] and Amira version 2020.2.

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Human like plexiform lesions identified in the prolonged Sugen5416/hypoxia rat model, visualised by synchrotron tomography imaging https://bit.ly/3KQvDHg

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The imaging revealed vascular pathology similar to human PAH. All four types of plexiform lesions previously identified in human PAH [1] were observed (figure 1). Segmentation of dye-filled vessels confirmed that plexiform lesions do communicate with the bronchial circulation in Sugen5416/hypoxia rats. As in human PAH, type 1 and type 2 plexiform lesions communicated with the vasa vasorum and peribronchial vessels, respectively. Neointimal thickening and complete arterial occlusions were present, but quite distal in the pulmonary vascular tree compared to human PAH. Type 4 lesions were observed, but as with the neointima, they were found in relatively small vessels. Patent IBAs without plexiform lesion formation were also observed (not shown).

In summary, SPµCT imaging of the prolonged Sugen5416/hypoxia rat model revealed patent IBAs, vascular remodelling and plexiform pathology similar to human idiopathic and familial PAH. Furthermore, it is our belief that the aneurysm-type plexiform lesions observed in the initial study [4] likely correspond to type 1 and 2 lesions in our material, as they were observed to originate from monopodial branches (supernumerary arteries). The prolonged model is very promising as it can be used to study the temporal and spatial regulation of PAH lesion formation, to establish a pathological timeline. Since the imaging method used here is non-destructive it can also be combined with subsequent sectioning and methods like immunohistochemistry and *in situ* hybridisation to decipher molecular mechanisms.

Although many novel pressure-lowering therapies have been introduced over the past two decades, no treatment currently available prevents the formation of PAH specific vascular pathology. We firmly believe that increased knowledge on the temporal aspects and underlying processes driving the formation of plexiform lesions, combined with insights regarding the haemodynamic roles of the different types of lesions, will bring us closer to developing novel treatments for the disease. This description of the vascular pathology of the Sugen5416/hypoxia rat is an essential first step in using the model to understand the biology of plexiform lesion formation and function and will hopefully be useful for many groups in the field.

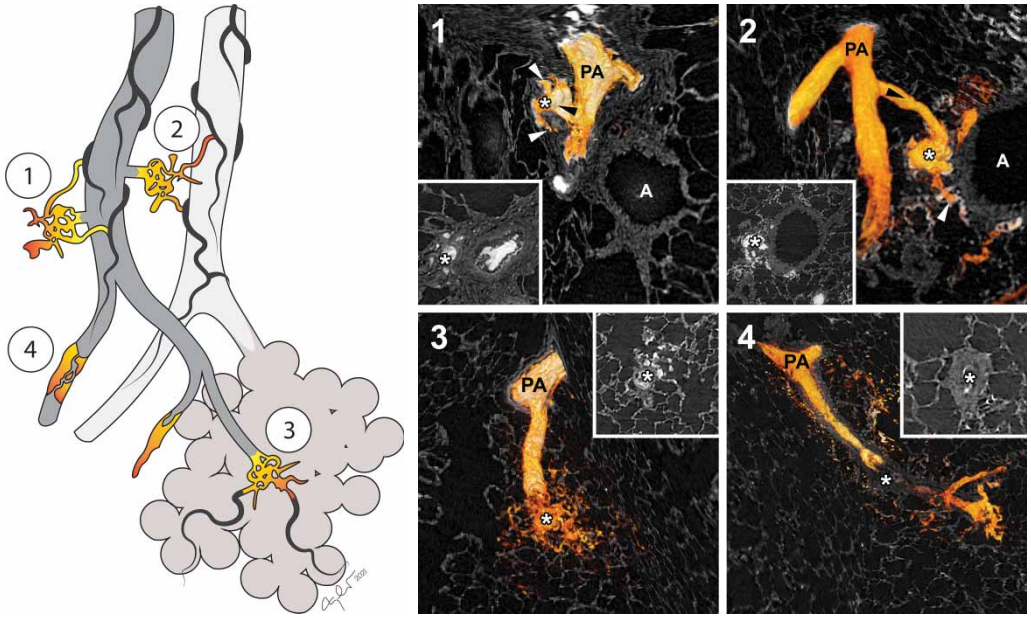

FIGURE 1 Three-dimensional reconstructions of the four types of plexiform lesions in the rat model. All four types of plexiform lesions previously described in human pulmonary arterial hypertension were identified (1–4) and the injected radiopaque dye was used to three-dimensionally reconstruct the vascular lumen of the different lesions. White asterisks mark the three-dimensional reconstructed plexiform lesions and, in insets 1–4, the same lesions in two-dimensional cross sections, as they would have been seen histologically. Type 1 lesions derive from monopodial branches/supernumerary arteries (black arrowhead), with connections to the vasa vasorum (white arrowheads). Type 2 lesion are best described as tortuous transformations of IBAs (black arrowhead), connected to peribronchial vessels (white arrowheads). Type 3 are found at abrupt ends of distal, intra-acinar, pulmonary arteries/arterioles and type 4 lesions are blocked pulmonary arteries/arterioles with re-canalisation. All four types of plexiform lesions are also illustrated in multi-dimensional video format (supplementary videos 1–4). PA: pulmonary artery; A: airway.

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