Plataus lauko antros harmonikos generacijos mikroskopija, skirta fibrozės progresavimo analizei esant arterinei plaučių hipertenzijai

Wide-field second-harmonic generation microscopy for analysis of fibrosis progression during pulmonary arterial hypertension

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Collagens are the key structural proteins in lung, responsible for the integrity of airways, bronchovesicular bundles, blood vessels, and alveolars. Its overproduction leads to fibrosis, often complicated with pulmonary arterial hypertension (PAH) – a syndrome caused by pulmonary arterial remodeling and leading to increased pulmonary vascular resistance, right ventricular hypertrophy, and heart failure. Conventional techniques for collagens imaging require several stages of preliminary tissue preparation and can cause undesirable morphological changes in the extracellular matrix, containing collagen. At the same time, second-harmonic generation (SHG) microscopy allows label-free imaging of collagen because of its non-centrosymmetric structure. In this study we demonstrate the application of recently developed wide-field SHG microscopy [1] for label-free investigation of fibrosis progression during PAH.

PAH in rats was chemically induced via monocrotaline injections. Tissue samples of healthy animals (control group) and animals with PAH on the 4th and 8th weeks of disease progression were investigated by a combination of brightfield, fluorescence, and SHG microscopies of hematoxylin-eosin (HE) stained samples. Imaging was performed on a home-build wide-field SHG microscope. Images of slices of lung tissue are presented in Fig. 1 with SHG signal colored in yellow and fluorescence in red. In control group, collagen can be seen to surround the blood vessel wall (marked with arrows in Fig. 1b) but is absent in the lung tissue. In rats with PAH, significant collagens overproduction is observed (Fig. 1d, f), while HE images (Fig. 1c, e) demonstrate only thickening of the walls of blood vessels as compared with control samples (Fig. 1a). In rats with PAH, collagens content increases in time-dependent manner, fibers are long, highly assembled, form dense fiber network both around blood vessels and in the surrounding tissue propagating deep inside the alveolar region (marked with arrows, Fig.1d, f). This supports our previous results [2], indicating a significant increase in the expression of collagens after 4-8 weeks of PAH progression.

Thus, SHG microscopy could serve as a powerful tool for label-free, fast, and sensitive analysis of fibrosis progression during pulmonary arterial hypertension.

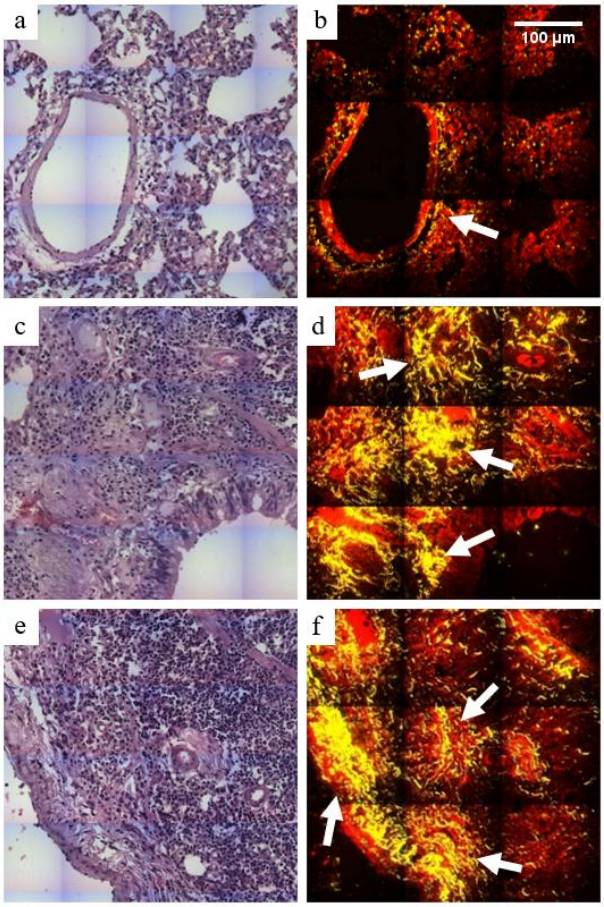

Fig.1. Brightfield images of HE-stained lung tissue (a, c, e), and merged fluorescence and SHG images (b, d, f) in: (a, b) – control group rats, (c, d) and (e, f) – rats on the 4th and the 8th week of PAH induction, respectively.

Keywords: second-harmonic generation, pulmonary arterial hypertension, fibrosis, collagen.

Literature

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