Contribution of CLEM to study the PAH-related pulmonary vascular remodeling

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Pulmonary Arterial Hypertension (PAH) is a rare disease featured by obstructive lesions of small pulmonary arteries (Ø<500µm). We demonstrated that Endothelial-to-mesenchymal transition participates to this vascular remodeling. Vimentin (Vim) is the main structural protein of the mesenchymal cell and its phosphorylation regulates its assembly/disassembly allowing the cell differentiation, proliferation and migration. Hence, increase in phospho-vimentin (Ph-Vim) could be a robust feature of pulmonary vascular remodeling in PAH. Even if Western-blot analysis performed on whole lung revealed a significant increase in Ph-Vim in PAH patients (26 fold compared to controls), confocal microscopy observations could not confirm this accumulation and assign it to a subcellular localization in vascular structures.
To address this problem, we used the correlative light and electron microscopy (CLEM) approach to observe vascular structures in confocal and transmission electronic microscopies. Cryosections (10µm) were immunolabelled with Phospho^{Ser55}-Vim antibodies, revealed by FluoroNanogold™ and examined with a confocal microscope. After glutaraldehyde post-fixation, gold amplification and Epon embedding, ultrathin sections (70 nm) were examined under TEM. Gold particles localized in artery endothelial and subendothelial cells were counted. This approach confirmed Western-blot results with significant increase in Ph-Vim in PAH patients (10.5 fold compared to controls) and segregation of Ph-Vim mainly in endothelial cells.
Hence, CLEM approach allowed demonstrating the implication of Ph-Vim in PAH vascular remodeling.