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

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1. INTRODUCTION

Pulmonary Arterial Hypertension (PAH) is a rare disease featured by obstructive lesions of small pulmonary arteries (Ø<500µm). Such lesions represent only 1% of the pulmonary tissue and we demonstrated that Endothelial-to-mesenchymal transition participates to this vascular remodeling [1]. 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.

2. RESULTS

Western-blot analysis performed on whole lung revealed a significant increase in Ph-Vim in PAH patients (26 fold compared to controls) but 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.

2.1 Methodology

The lung, a soft organ consisting of air-filled alveoli, is delicate to prepare for microscopy and needs specific treatments. Briefly, cryosections (10µm) were immunolabelled with Phospho^{Ser55}-Vim antibodies, revealed by FluoroNanogold™ [2] and examined with a confocal microscope. After glutaraldehyde post-fixation, gold amplification and Epon embedding, ultrathin sections (70 nm) were examined under TEM.

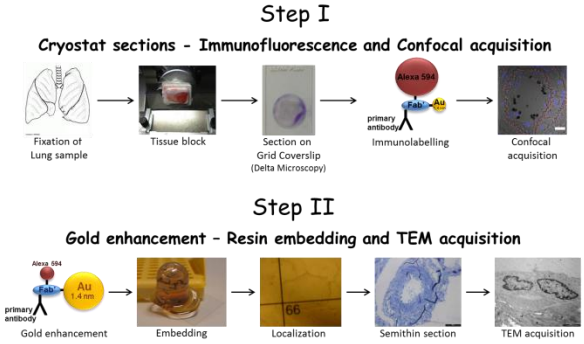

Figure 1: CLEM methodology applied to lung.

2.2 Structure and antigens preservation

In contrast to cryopreservation, this lung adapted methodology allows to preserve the tissue structure closed to results obtained with Epon embedding. Moreover, such tissue fixation and embedding allow the antigens preservation without producing non-specific labeling.

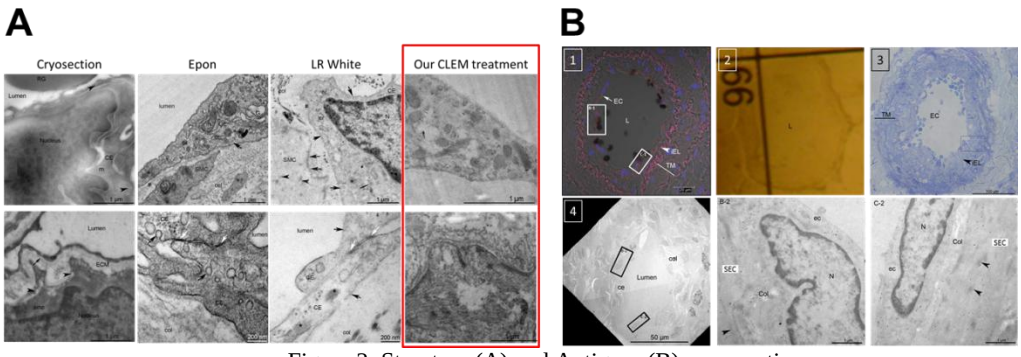

Figure 2: Structure (A) and Antigens (B) preservation

2.3 CLEM analysis and quantification

Confocal microscopy analysis was not resolutive enough to specifically identify and quantify Ph-Vim labeling in pulmonary lesions. Correlated to electron microscopy, this approach confirmed Western-blot results with significant increase in Ph-Vim in PAH patients (10.5 fold compared to controls) and demonstrated a segregation of Ph-Vim mainly in endothelial cells (EC) compared to smooth muscle cells (SMC).

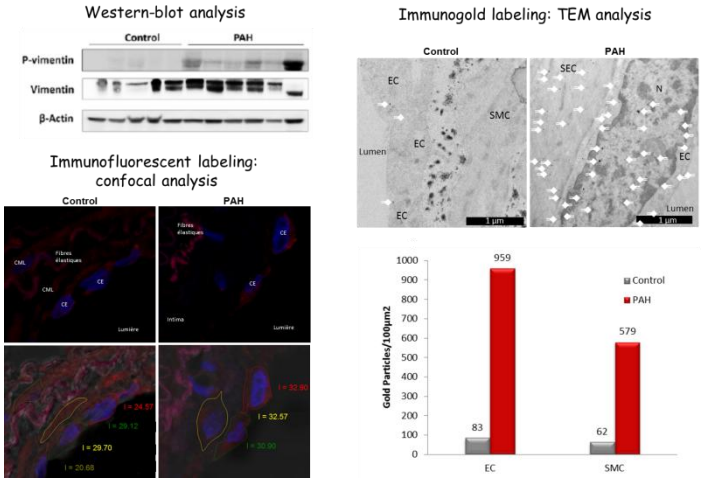

Figure 3: Western-blot, confocal and TEM analysis of Ph-Vim expression in PAH tissue

3. CONCLUSION

Hence, using correlative microscopy, we overcame the difficulties associated to the lung ultrastructure which is difficult to preserve during EM techniques (hydrophilic resins, Cryo-TEM) and to the location and identification of the small arteries of interest. We found out a good compromise that allowed us to evidence the presence of Ph-vim in EC and SMC in rare pulmonary lesions, little or not visible in confocal fluorescence microscopy. The CLEM approach allowed demonstrating the implication of Ph-Vim in PAH vascular remodeling.

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