RunX2 signaling drives pulmonary artery calcification and stiffening in pulmonary hypertension due to left heart disease

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Introduction: Pulmonary hypertension due to left heart disease (PH-LHD) is the most frequent cause of pulmonary hypertension and has a poor prognosis. Vascular calcification is a common pathological feature in aging and in cardiovascular diseases such as hypertension, diabetes mellitus, or atherosclerosis. Vascular calcification mainly manifests as increased vascular stiffness and decreased compliance. Here, we addressed the potential role of pulmonary artery (PA) calcification in vascular stiffening in PH-LHD. **Method and results:** We explored PA calcification in tissue samples collected perioperatively from PH-LHD patients during heart transplantation and in a rat model of PH-LHD induced by aortic-banding (AoB). Genome-wide RNA sequencing of PH-LHD patient and AoB rat PAs, followed by gene ontology (GO) term analysis identified significant enrichment in genes associated with ossification (GO:0001503) and osteoblast differentiation (GO:0001649), including increased expression of runt-related transcription factor-2 (RunX2), a master transcription factor in osteogenesis. PA calcification in both human proximal PA, and rat proximal and distal PAs was confirmed by Alizarin red and von Kossa staining, with percentage of calcified PA area in PH-LHD patients and rats exceeding corresponding controls by four and two folds, respectively. Similarly, colorimetric detection of calcium content in both human PAs and rat lungs identified a significant increase in Ca^{2+} levels in PH-LHD samples as compared to corresponding controls. Calcium content correlated with PA wall stiffness assessed *ex vivo* by uniaxial tensile test and with pulmonary hemodynamics measured by right heart catheter *in vivo* (mean pulmonary arterial pressure in patients or right ventricular systolic pressure (RVSP) in rats). Immunoblotting analyses demonstrated a nearly 1.5-fold upregulation of RunX2 and increased expression of bone markers alkaline phosphatase and Osterix in PAs of PH-LHD patients and lungs of AoB rats. Further immunofluorescence staining of isolated primary PA smooth muscle cells (PASMCs) revealed nuclear translocation of RunX2 in PH-LHD cells, when compared to controls. Concomitantly PH-LHD PASMCs showed enhanced susceptibility to calcification induced by culturing in phosphate/calcium enriched medium. The role of RunX2 in PA calcification as a pathomechanism in PH-LHD was tested *in vivo* by inhibition of RunX2. In AoB rats, RunX2 function was silenced by repeated intraperitoneal injections of RunX2-DNA binding inhibitor CADD522. In comparison to vehicle treated controls, CADD522 application reduced PAs calcification in AoB rats and decreased RVSP, PA wall thickness, and right ventricular hypertrophy, indicating PH attenuation. **Conclusion:** Our study demonstrates PA calcification in patients with PH-LHD that correlates with pulmonary hemodynamics and PA stiffness. RunX2 activation and PA calcification emerge as important determinants of PA stiffness and PH severity in PH-LHD. Targeting this axis may provide a new potential therapeutic strategy for patients with PH-LHD.

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