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# Cytokine induced downregulation of *Plasma Membrane Calcium ATPase 4* gene increases sensitivity to apoptosis in pulmonary artery endothelial cells

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**INTRODUCTION:** Pulmonary arterial hypertension (PAH) is a life-threatening disease characterized by progressive vasoconstriction, vascular remodelling, and occlusion of small pulmonary arteries. It leads to increased pulmonary resistance and finally right ventricular failure. The disorder has no cure and current therapies only target vasoconstriction but have little effect on vessel remodelling. Increased activity of pro-inflammatory cytokines is linked to PAH pathogenesis. In this study, we analysed the effect of TNF- $\alpha$  and IL-1 $\beta$  on the expression of Plasma Membrane Calcium ATPase 4 (PMCA4) in pulmonary artery endothelial cells (PAEC).

**METHODS:** PAEC were cultured for different times and with different doses of TNF- $\alpha$  or IL-1 $\beta$ . Expression of *PMCA4* RNA and protein was determined by qPCR and western blot respectively. *PMCA4* expression was silenced using siRNA specific for human *PMCA4*. Quantification of apoptotic cells was performed by flow cytometry and TUNNEL.

**RESULTS:** Treatment of PAEC with TNF- $\alpha$  or IL-1 $\beta$  induced a time- and dose-dependent decrease in the levels of RNA for *PMCA4*. Analysis of *PMCA4* RNA levels in the lungs of mice with overexpression of ectopic TNF- $\alpha$  confirmed the *in vivo* relevance of these observations. RNA decay experiments performed by blocking cellular transcription with Actinomycin D indicate that the downregulation of *PMCA4* RNA levels mediated by pro-inflammatory stimuli in PAEC is the result of a decrease in RNA stability. In agreement with the reduction observed in RNA levels, *PMCA4* protein expression was strongly decreased by treating PAEC with TNF- $\alpha$  or IL-1 $\beta$ . Silencing *PMCA4* gene expression sensitised PAEC to apoptosis, suggesting that *PMCA4* protects PAEC to apoptosis induced by pro-inflammatory cytokines.

**CONCLUSION:** The pro-inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  significantly downregulate the expression of the *PMCA4* gene in PAEC at the RNA and protein level. Decrease in *PMCA4* expression sensitised PAEC to apoptosis. This indicates that the *PMCA 4* gene might play an important role in the apoptotic loss of endothelial cells observed in the pulmonary arterioles of patients with PAH.