Abstract: P6011

NKX2-5 contributes to EndoMT and endothelial dysfunction in pulmonary arterial hypertension

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Background: The onset of inflammation, hypoxia or shear stress within blood vessels can result in endothelial-to-mesenchymal transition (EndoMT), a disease-associated process where endothelial cells (ECs) downregulate endothelial markers and acquire mesenchymal features. EndoMT is observed in patients with scleroderma-associated pulmonary hypertension (SSc-PAH), which have the highest mortality amongst all the scleroderma patient subgroups. The homeobox transcriptional factor NKX2-5 is fundamental for cardiovascular development. However, NKX2-5 expression has not been reported yet in ECs of adult pulmonary blood vessels.

Purpose: To investigate the role of NKX2-5 in the pulmonary endothelium of SSc-PAH.

Methods: Human pulmonary artery endothelial cells (HPAECs) were treated with a cocktail of TGF-β (5 ng/mL), TNF-a (5 ng/mL), and IL-1β (0.1 ng/mL) for 5 days. Immunofluorescence was used to detect NKX2-5 and other markers in ECs. Western blotting and qPCR evaluated, respectively, protein and gene expression. Lentiviral transduction forced NKX2-5 expression in the cells. Transendothelial electrical resistance (TEER) measurements evaluated endothelial barrier function. Pharmacological inhibition was performed to determine the pathways that lead to NKX2-5 activation. Casein kinase 2 (CK2)-inhibition (CX4945) of a chronic hypoxia mouse model of PAH was used to assess right ventricular systolic pressure (RVSP).

Results: Immunofluorescence showed a strong expression of NKX2-5 in the endothelium of SSc-PAH human lungs (p<0.0001). Western blot analysis demonstrated a 5.3-fold downregulation of CD31 (p<0.001), and an increased production of NKX2-5 (5.6-fold, p<0.0001) and of Procollagen I (12-fold, p=0.0009) after 5 days of cytokine stimulation on HPAECs. Relative mRNA expression has shown a 3-fold gene downregulation of CD31 (p=0.0002) and a reduction of VE-Cadherin (2.3-fold, p=0.0008) and of vWF (10.4-fold, p=0.003) in EndoMT, whereas gene expression of COL1a2 (8.5-fold, p=0.0001) and of NKX2-5 (1.5-fold, p=0.003) were upregulated. Immunofluorescence of cells has revealed a decreased VE-Cadherin expression concomitant with upregulation of NKX2-5 in EndoMT cells. Forced expression of NKX2-5 downregulated endothelial markers and endothelial barrier function was impaired whereas proliferation rate of cells was increased. Inhibition of PI3K, ERK5, ALK5 and CK2 reduced NKX2-5 protein expression within cells. CK2-inhibited mice under hypoxia conditions resembled the normoxia mice group by normalising RVSP.

Conclusion: HPAECs undergoing EndoMT express NKX2-5 in vitro and in vivo, via mediation of CK2, TGF-β, ERK5 and PI3K signalling. NKX2-5 downregulates key adherence junctional proteins, disrupting endothelial barrier function. This study highlights the involvement of NKX2-5 in EndoMT and in endothelial dysfunction, leading to vascular disease progression in SSc-PAH.