In Vivo Knockdown of Snail is a Novel Therapeutic Strategy for Right Ventricular Failure

Presenting Author: Varina R. Clark, BS

Institution: David Geffen School of Medicine at UCLA

Contributing Authors: Nicole Yin, MD, Somanshu Banerjee, PhD, John Park, PhD, Michael Zargari, Emma Said, Darnell Bagsik, Greg Fishbein, PhD, Louis Saddic, MD, PhD, Soban Umar, MD, PhD; David Geffen School of Medicine at UCLA

Abstract body

Background

Pulmonary hypertension(PH) induces RV-failure(PH-RVF) by poorly understood mechanisms. We hypothesized that PH-RVF is associated with Endothelial-to-Mesenchymal Transition(EndMT) driven by transcription factor Snail and Snail knockdown may rescue PH-RVF in rats.

Methods

PH-RVF was induced in male SD rats(250-300g) with single s.c. injection of Monocrotaline(MCT,60mg/kg,n=4;30-days) or Sugen(SU5416 20mg/kg,n=4;10%O2-hypoxia 3-weeks+normoxia 2-weeks). Saline-treated rats served as controls(CTRL, n=4). For in-vivo Snail-knockdown, MCT-rats received Snail-siRNA(n=6; 5nM/injection q3-4 days; 4-injections) or scramble(n=7) from day 14-30 after MCT. Echocardiography and RV-catheterization were performed terminally. RVs were stained with Trichrome and EndMT markers. RV gene expression was assessed by RNASeq and validated with RT-PCR. Human RV sections(CTRL n=3, PAH n=7) were stained for Snail. Values are mean±SEM.

Results

PH-RVF was confirmed by increased RVSP(MCT:97.6±6.6mmHg, Su/Hypoxia:85.26±15.6 vs CTRL:37.1±1.3; $p<0.05$), RV-hypertrophy (RV/LV+IVS: MCT:0.82±0.07, Su/Hypoxia:0.61±0.1 vs CTRL:0.27±0.01; $p<0.05$) and RV-dilatation (RVID-diastolic: MCT:3.5±0.3mm, Su/Hypoxia:2.5±0.2 vs CTRL:1.3±0.1; $p<0.05$). PH patients had significantly elevated RVSP(80.7±9.9 mmHg) and reduced RV-function. PH-RVF rats and patients demonstrated RV-fibrosis and EndMT (co-localization of endothelial/smooth muscle markers). RNASeq demonstrated EndMT as the top upregulated pathway in both MCT and Su/Hypoxia RVs. Snail was significantly increased(~2-fold) in MCT and Su/Hypoxia($p<0.05$) RVs whereas other known EndMT-inducing transcription factors Snai2, Twist1, and Zeb1 were unchanged. Human RVs also demonstrated increased nuclear immunolabeling(activation) of Snail. MCT-rats treated with Snail-siRNA demonstrated decreased RV Snail expression, RVSP(45±1.6 vs 60±3.89 mmHg; $p<0.05$), RV hypertrophy(0.41±.01 vs 0.81±.06; $p<0.05$) and improved RV-function.

Conclusions

Pre-clinical and clinical PH-RVF is associated with EndMT mediated via Snail and its network. Targeting Snail is a novel therapeutic strategy for PH-RVF.