Title: Pulmonary venous hypertension and mechanical strain stimulate monocyte chemoattractant protein-1 release and structural remodelling of the lung in human and rodent chronic heart failure models

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On-line Supplement

Western blotting: Equal amounts of protein (20µg) were loaded into a 10% SDS-polyacrylamide gel followed by electrophoresis. Protein samples were mixed with a sample buffer, boiled for 10 minutes, separated by SDS-PAGE under denaturing conditions, and electro-blotted onto nitrocellulose membranes. The blots were incubated overnight in Tris-buffered saline (TBS) containing 5% milk to block unspecific binding of the antibody. Proteins of interest were revealed with specific antibodies as indicated (1:1000 dilution) for 1 hour at room temperature followed by incubation with a 1:5000 dilution of horseradish peroxidase-conjugated polyclonal anti-rabbit antibody for 1 hour at room temperature. Signals were visualised by chemiluminescent detection. Equal protein loading of the samples was further verified by staining the total amount of p42/44 ERK.

	AMC	Sham	p
Body Weight (g) n=9	431.1 (±32.5)	417.4 (±44.5)	0.44
HW:BW (n=9)	3.51 (±0.3)	3.74 (±0.4)	0.17
LW:BW (n=9)	4.54 (±0.7)	4.8 (±0.6)	0.28
Serum MCP-1 (n=6)	76.8 (±26.3)	75.3 (±12.5)	0.65

Table 1: Online Data Supplement: Parameters from heart failure rodents at 16 weeks post myocardial infarction (HF) or age matched controls (AMC). n the number of animals in each group.

Abbreviations: HW- heart weight, BW- body weight, LW- lung weight.

Student's t-test, mean (± SD).

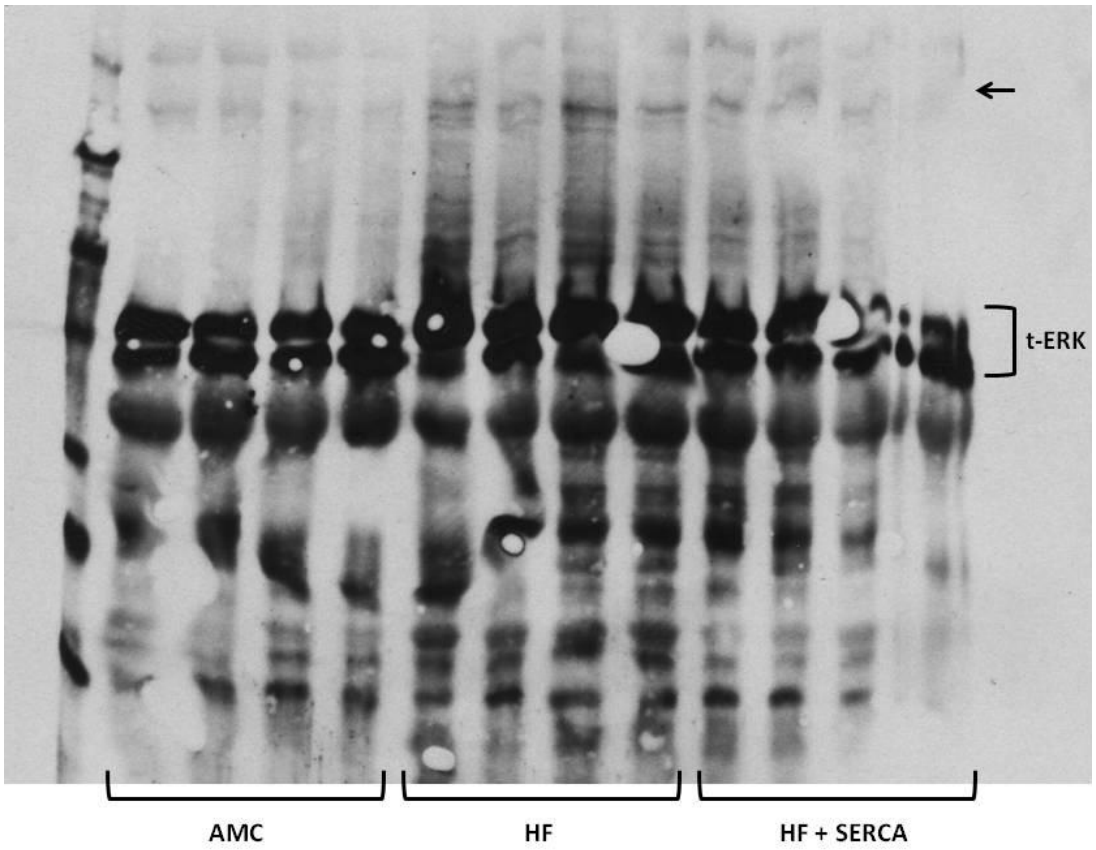

Figure 2: Whole rat lung protein from age matched control animals (AMC), post myocardial infarction (HF) and post myocardial infarction animals treated with SERCA2a gene therapy (HF + SERCA) did not show the presence of SERCA2a (arrow, 100 kDa) but did show the presence of total p42 and p44 ERK (t-ERK: to confirm protein loading).

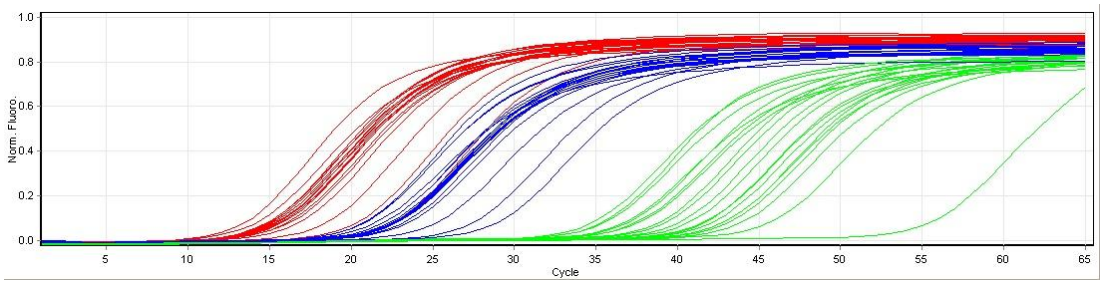

Figure 3: SERCA2a (green) gene expression was not seen, or seen at very low levels, compared to the housekeeping gene ARBP (red) and SERCA2b (blue) as demonstrated by the markedly lower cycle time amplification at PCR. Plots represent samples from whole lungs of age matched control, post myocardial infarction and post myocardial infarction with SERCA2a gene therapy animals.