Statin therapy transforms cardiac fibroblast function in human failing heart

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**Problem**
The effect of statin therapy, a commonly used lipid lowering strategy in patients at risk for cardiovascular disorders, on cardiac fibroblast function is not known.

**Background**
Cardiac fibrosis underlies in the progression of atrial fibrillation and heart failure. Fibroblast proliferation and differentiation precede fibrosis. Statins (HMG-CoA (3-hydroxy-3-methylglutaryl-Coenzyme A) reductase inhibitor) therapy is recommended (by ACC/AHA) for patients having cardiovascular disease. Apart from the established lipid lowering effects, statins have other effects reported in animal models but its effect on human ventricular fibroblasts (HVF), responsible for extracellular matrix secretion and fibrosis, is unknown. As excessive fibrosis is associated with heart failure (HF) and fibroblast-myofibroblast trans-differentiation precede fibrosis, we tested the hypothesis that statin therapy interferes with the normal proliferation and differentiation function of HVFs from HF patients.

**Objective**
To determine the effect of statin therapy on cardiac fibroblasts, isolated from failing heart patients either under statin therapy or not, and to determine the signaling mechanisms involved in this effect.

**Methods**

**Cell Culture**
Primary cultures of HF from HF patients undergoing (VAD) implantation under statin therapy (HF+Statin) for at least 1 year (n+) or not (n-), non-diseased HF from trauma victims (n=3), were compared.

**Proliferation assay**
The fibroblast proliferation was assessed by 5-ethyl-2-deoxyuridine (EdU), a thymin analogue, incorporation assay, and cell counts using hemocytometer.

**Immunobleching**
Expression of a smooth muscle actin (α-SMA) and GAPDH was assessed in fibroblast culture lysate.

**Immunohistochemistry**
Myofibroblasts were identified with immunostaining of a-SMA using appropriate primary/secondary antibodies, visualized under confocal microscopy and quantified using Fiji software.

**PCR Array**
Transcriptomic changes were studied from total RNA using RT2 Profiler™ PCR array.

**Statistical Analysis**
Unpaired Student’s t-test or one-way ANOVA

**Results**

**Statin Therapy Mitigates Fibroblast Differentiation**
Fig. 1: Representative western blotting images(top) of HVF lysates probed for the expression of α-SMA. Failing heart HVFs that were NOT under statin therapy showed significantly higher expression of α-SMA compared to the failing heart HVFs that were under statin therapy for at least one year. Bar graph(bottom) displays the pooled average image densities of α-SMA bands normalized to the corresponding GAPDH bands. Bands were quantified using Image J software. Y=0.03 vs Control, P=0.01 vs HF+Statins

**Statin Therapy Alters the Transcriptionome of Cardiac Fibroblasts**
Fig. 5: Transcriptomic changes were studied from total RNA using RT2 Profiler™ PCR array. Data were analyzed by Student’s t-test. Among the 84 transcription factors (TF) profiled, statin therapy upregulated significantly the following 15s: CREB1, SMAD1, TCF1, LIF, HIF2A, ATF3, and SFP3 that are mainly involved in signaling pathways of GPCRs, bone morphogenetic proteins, Wnt, MAPK/ERK, and miscellaneous pathways, respectively. TAP2A2 tends to be downregulated by two-fold, but not statistically significant.

**Statin Therapy De-Differentiates Myofibroblasts to Fibroblasts**
Fig. 4: In vitro treatment of simvastatin (300 nM for 72 hrs) on HF- No_Statinsignificantly reduced (reversed) the high expression of α-SMA (Diff_HVF). * P<0.05 compared to all other groups.

**Conclusion**
Statin interferes in the human ventricular fibroblasts differentiation function by associated changes in the transcriptome and signaling molecules involved in fibrosis. This anti-fibrotic effect of statins may be harnessed in therapeutics to mitigate the progression of cardiac fibrosis and heart failure, apart from its lipid-lowering effect.