Promoting Neonatal Cardiomyocyte Proliferation using a Cryptic Matrikine Derived from Fibrillin-1

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Introduction: We have previously shown that fetal, ventricular, cardiac ECM (cECM) promotes neonatal cardiomyocyte proliferation. However, the specific peptides that promoted this effect have yet to be identified. The hypothesis of this study was that there exists a cryptic peptide or combination of peptides within cECM that led to this enhanced cardiomyocyte proliferation and that these peptides are either promoted in early cardiac development or repressed in aging by other peptides or biological macromolecules.

Methods: Following decellularization of adult and fetal Sprague Dawley rat hearts (Fig. 1A), samples were fractionated according to molecular weight using SDS-PAGE. Half of the resulting gel was transferred to a PVDF membrane and cardiomyocytes (CMs) were cultured in monolayer directly on the PVDF membrane (Fig. 1B). The other half of the gel was saved for subsequent analysis of composition of the cECM fraction via LC/MS-MS proteomics. Membrane cultures of CMs were analyzed using cell profiler for proliferation and regions of high CM proliferation were identified. Corresponding refgions in the saved half of the gel were

Results: CMs cultured directly on the PVDF membranes with fractionated cECM (5 days in serum-free media) revealed specific bands of cECM with enhanced proliferation (Fig. 1C), and corresponding proteomics analysis of these molecular weight regions in the stored gel fragments implicated several peptides near the N-terminus of fibrillin-1 (Fig. 1D). The first of these regions, denoted the "F1R1" peptide (residues 55-86), was fabricated and adsorbed onto TCPS. CMs cultured in serum free conditions on F1R1 demonstrated significantly enhanced proliferation over both positive (TCPS/serum media) and negative (PLL, serum-free media) controls ($p<0.05$) at conditions of 6 mg/ml and 10 mg/ml (Fig. 1E). Scrambled F1R1 (S) and linearized F1R1 (A) (alkylation of cysteine residues to prevent folding) did not elicit CM proliferation. These data suggest that the N-terminus of fibrillin-1 may play a role in CM plasticity in the developing myocardium and could have utility as a novel therapeutic for cardiac re-generation. Future work includes evaluating the effect of the F1R1 peptide on adult CMs and cardiac progenitor cells, as well as tests of in vivo efficacy.

Conclusions: The findings of this study demonstarte enhanced CM proliferation as a result of a 5-day culture upon peptides derived from fractionated cECM. Synthesis of matrikines identified from Fibrillin-1 maintains the proliferative potential of neonatal cardiomyocytes and this proliferation is abolished when the peptide is scrambled or linearized. Future work aims to isolate and characterize other matrikines present in cECM, to assess the effects of these and other matrikines on cardiac stem cell differentiation and adult cardiomyocyte proliferation and to utilize these matrikines in an in vivo model of cardiac repair such as myocardial infarction.

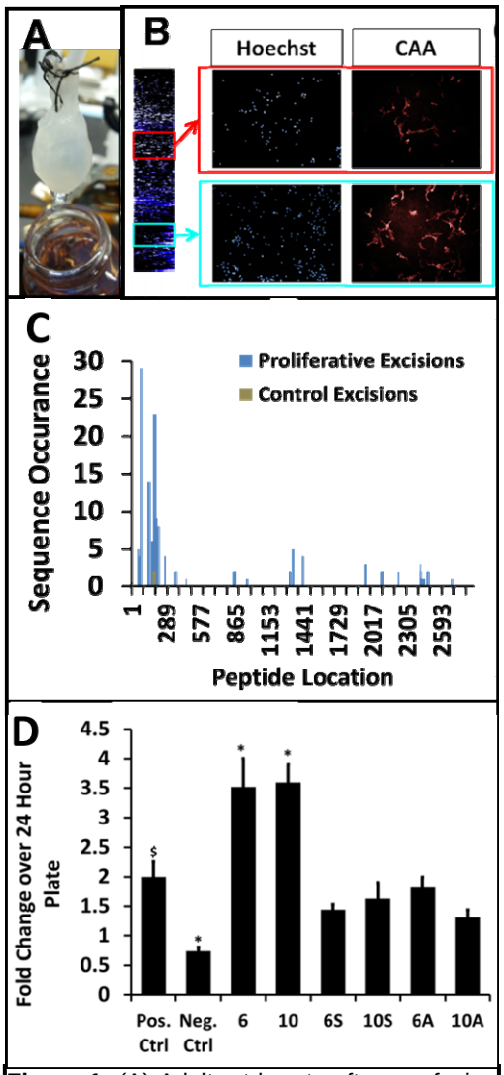

Figure 1: (A) Adult rat hearts after perfusion decellularization. (B) Example images for entire PVDF lane (left) and blow ups of specific regions (right) (CAA= cardiac α-actin for CMs). (C) Mapping of peptides derived from LC-MS/MS of proliferative and control regions into the Fibrillin-1 protein. Note the peaks between residues 55 and 234. (D) Fold change in CM number at 5 days on 6 and 10 μg/ml of F1R1 peptide, scrambled F1R1 peptide (S), and Alkylated F1R1 (A). *p<0.05 w.r.t all other groups. $ p<0.05 w.r.t. negative control.