Mechanisms of contractile dysfunction in lamin A/C-deficient hearts

D. Fatkin, Sr Bernice Research Program in Inherited Heart Diseases, Molecular Cardiology Division, Victor Chang Cardiac Research Institute, Darlinghurst, NSW 2010, Australia.

Mutations in the *LMNA* gene that encodes the nuclear lamina proteins, lamin A and lamin C, have been associated with diverse human disorders and are the most common cause of familial dilated cardiomyopathy (DCM). The mechanisms by which nuclear protein defects result in cardiac contractile dysfunction are incompletely understood. Mice with a targeted deletion of the *Lmna* gene develop DCM and are a useful model to study disease pathogenesis. Homozygous *Lmna* knockout ($Lmna^{-/-}$) have severe DCM and die by 6-8 weeks of age, while heterozygous ($Lmna^{+/-}$) mice develop a milder phenotype in adult life with reduced survival after 40 weeks. Previous studies have established that lamin A/C-deficient nuclei have increased deformability and reduced survival when subjected to biaxial strain. On the basis of these findings, it has been proposed that mechanical stress-induced apoptosis contributes significantly to the development of DCM in patients with *LMNA* mutations. The aim of our study was to test this "structural hypothesis" by determining the effects of interventions to modify mechanical stress in $Lmna^{+/-}$ mice. Serial echocardiography and tissue studies were performed in wildtype (WT) and $Lmna^{+/-}$ mice before and after exercise training, thoracic aortic constriction (TAC), and administration of a β-adrenergic receptor-blocking drug, carvedilol. Echocardiography was performed in anaesthetized mice (avertin 2.5%). Mice were ventilated and anaesthetized for surgical procedures with ketamine (75 mg/kg), xylazine (20 mg/kg) and atropine (0.6 mg/kg). Tissue analyses were performed post-mortem in excised hearts. We first evaluated the biophysical properties of isolated cardiomyocytes from mice aged 12 weeks (prior to DCM) and found changes in nuclear size and shape in $Lmna^{+/-}$ mice, as well as altered distribution of perinuclear desmin and enhanced swelling responses to hypo-osmotic stress. Groups of 12 week-old WT and $Lmna^{+/-}$ mice underwent a 6-week exercise training program. Contrary to our predictions, neither moderate nor severe intensity exercise training induced cardiomyocyte apoptosis (assessed by TUNEL assay and caspase-3 activation) or DCM. Sustained left ventricular pressure overload generated by TAC resulted in apoptosis and contractile dysfunction in WT and in $Lmna^{+/-}$ mice, with no differences in severity between the genotype groups. We found however, that regular moderate exercise training attenuated DCM development in male $Lmna^{+/-}$ mice. Oral administration of carvedilol from 6 weeks to 40 weeks also had a protective effect against DCM in male $Lmna^{+/-}$ mice.

These data indicate that lamin A/C-deficient cardiomyocytes have intrinsic structural defects but that mechanical stress-induced apoptosis is not a major determinant of DCM. We propose that altered cytoskeletal stability due to loss of normal nuclear anchoring may impair force transmission and promote DCM in $Lmna^{+/-}$ hearts. Exercise training and carvedilol administration from an early age are promising strategies for DCM prevention and warrant further clinical evaluation.