Bi-Phasic Remodeling in a Murine Heart Failure Model

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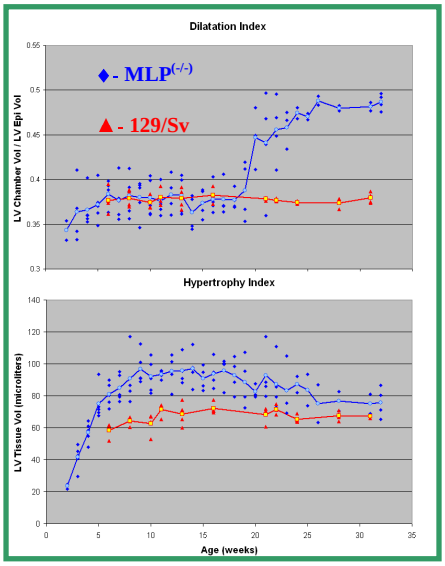
Introduction: The availability of gene-targeted mouse models has increased demands for high field murine cardiac MR imaging. The ability to serially track a genetically engineered model non-invasively with high spatial and temporal resolution will be valuable for understanding function and anatomy at both a tissue and whole organ level. Mice that are homozygous null for muscle LIM-domain protein (MLP) exhibit clinical characteristics of dilated cardiomyopathy, with systolic function depressed after two weeks of age, and continued progression towards congestive heart failure[1]. This pattern of anatomical and functional remodeling in the MLP knockout ($MLP^{(-/-)}$) heart may have useful clinical implications because mutations in the MLP gene have been identified in a subset of patients with heritable DMC. The goals of this work were to investigate the time course of anatomical remodeling in this mouse model of heart failure and identify critical time points that may help elucidate the pathogenesis of ventricular dilatation and failure.

Methods: MLP is thought to play an important role in proper cardiomyocyte architectural organization within the heart wall. It has also been proposed that MLP is a scaffold protein on the actin-based cytoskeleton responsible for sensing mechanical load on the myocyte and mediating mechanotransduction. Five $MLP^{(-/-)}$ and four non-failing wildtype controls (129/Sv) were imaged weekly from 2-3 to 32 weeks of age under an identical protocol and conditions. *In vivo* NMR murine cardiac imaging was performed on a 7T horizontal-bore MR scanner (Varian, Palo Alto, CA), equipped with a shielded 12 cm bore gradient system (22 G/cm, risetime 300 μs) (Magnex Scientific, Oxford, UK), and a custom designed 19 mm quadrature driven TEM coil. During scanning, mice were anesthetized with 1.5 Vol-% isoflurane, monitored for temperature (35-37°C) and ECG (450-550 BPM). High resolution MR experiments were conducted using an ECG and respiratory triggered Fast Low Angle SHot (FLASH) Gradient Echo (GE) pulse sequence (α=90°, TE=1.8ms, TR~R-R interval, 5 avgs) employing fractional echo and time series averaging. Seven to nine 1mm thick short axis slices were collected (apex to base) with a FOV of $25mm^2$ and data matrix of 128^2 yielding an in-plane resolution of $195\mu m^2$. Images were acquired at a range of time delays relative to the ECG trigger to isolate end-diastolic and end-systolic frames with a temporal resolution of 10ms. Manual segmentation of cardiac surfaces provided the geometrical data for analysis.

Results: ECG and respiratory gated MRI provide images of high enough spatial and temporal resolution to measure left ventricular chamber volume and wall thickness at end-diastole and end-systole. Figure 1 illustrates two calculated indices that describe the time course of remodeling in mutant and control mice: a Dilatation Index, defined as the LV chamber volume divided by the LV epicardial volume, is a three dimensional analog to a radius/wall thickness ratio, and a Hypertrophy Index, calculated as the LV epicardial volume minus the chamber volume, measures tissue volume in microliters. An early phase of rapid growth is evident in the failing hearts prior to six weeks of age, where physiological growth is probably supplemented with pathologic growth. Wall volume peaked near week 11 in the knockout mice but then declined throughout the remaining weeks. Dilatation in the failing hearts did not begin until near week 18, where it sharply increased and plateaued again near week 25. It appears that the peak in tissue volume temporally precedes the abrupt dilatation phase. In addition, systolic function was seen to improve temporally concurrent with the dilatation phase, as evidenced by an increase in stroke volume and ejection fraction (data not shown).



Discussion: To date, the structural characterization of remodeling in a failure model has not been achieved with a temporal resolution on the order of weeks. Non-invasive MR imaging techniques have made such longitudinal studies possible. The use of appropriate geometrical indices on data obtained from the same animal allows each to serve as its own normalizing factor, making it possible to measure a significant and reproducible change in anatomical structure over time. The two indices described above demonstrate a bi-phasic remodeling response in this failure model. The peak in tissue volume, and subsequent increase in volume loss, temporally precedes any dilatation, a known marker of the transition towards overt failure. It is proposed that the inability to further maintain hypertrophic remodeling mediates the onset of dilatation and eventual congestive heart failure[2]. Therefore, understanding the contributing factors to the decrease in tissue volume may uncover potential initiating factors to this transition. It is hypothesized that a balance exists between cell growth/atrophy and apoptosis that may shift in favor of programmed cell death near the peak in tissue volume. The MLP knockout mouse thus presents a reproducible and distinct time course of anatomical remodeling, where early hypertrophic growth exceeds that of normal controls. The process becomes decompensated in coincidence with a sudden onset of dilatation well after systolic dysfunction is first detected. Therefore, longitudinal MR imaging of high temporal resolution, in conjunction with a failure model whose characteristics have strong clinical implications, suggest that alterations in hypertrophic remodeling may mediate the apparent transition towards overt heart failure.

References: 1. Arber, S. (1997) *Cell*; 88(3):393-403.

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