Non-sarcomeric causes of heart failure: a Sydney Heart Bank perspective

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The Sydney Heart Bank (SHB) has contributed and continues to contribute samples of human left ventricles to our understanding of molecular mechanisms underlying heart failure (Li et al. 2013; Li and dos Remedios 2015). The great majority of the publications concern myofibrillar proteins. To date, the SHB has published about 120 research papers with about 60 collaborators around the world. About 20% of them involved genes and proteins that did not involve sarcomeric proteins.

Working with the SHB

The authors took the position that we should not expect to identify all of the causes of heart failure mechanisms, and that the best way forward would be to collaborate with the many laboratories in searching for an answer. The major obstacle to this aspiration was the need to convince these labs that your heart samples were unique in several ways. First we had to dispel the notion that our samples were degraded, or at least partially degraded due to the time between harvesting and preservation in liquid nitrogen. That obstacle would only be dispelled by sending out samples and wait for the results. This was particularly true for the donor hearts.

Minimal delay collecting donor hearts

Many cardiac research laboratories assumed that the non-failing donors (some wrongly called them “controls”) were

obtained post-mortem, i.e., obtained 12–24 h after death. In fact, our donors come from healthy individuals on life support that are harvested by the heart transplant surgeons soon after the declaration of brain death by the transplant coordinators. They were rejected by the transplant surgeons for a range of reasons, commonly because no tissue-type match could be found in the heart failure clinic at St Vincent’s Hospital, but sometimes, the hearts were suspected of having coronary vascular disease, but usually this was dispelled once we began sectioning the heart before freezing. Sometimes, the hearts were simply too young.

All donor hearts were quickly perfused with ice-cold cardioplegia that rapidly chilled the heart and arrested the heart-beat. When the valves were required for transplantation, the hearts were rapidly transported to St Vincent’s Hospital in Sydney. When a donor was a juvenile or infant, the valves were not required and were delivered to the SHB team. Infant and juvenile hearts were obtained very precious and, thanks to the care and diligence of the clinical teams in participating hospitals particularly at the John Hunter Hospital in Newcastle. We are very grateful to those families of those very young donors who hoped some good would come from the death of their children.

This bank of non-diseased donors turned out to be more sought-after than the failing hearts. Today after nearly 30 years, we have assembled a substantial bank of hearts where the older heart groups were used as age-matched (and sex-matched) donors for the failing hearts. Figure 1 shows the age distribute of the donor hearts. The SHB currently has 120 of these hearts.

The failing hearts were also preserved in liquid nitrogen (–194 °C) as close as possible to the time when they were removed from the transplant patient. They remain perfused by the cardiac by-pass pump until the moment the aorta was cross-clamped. Within 2–3 min, the patient aortic and pulmonary semilunar valved were removed by the Sydney Valve Clinic personnel and the first 10–20 g samples of left ventricle free wall were frozen within 5–15 min. This speed of

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Fig. 1 The age distribution of the non-diseased donor hearts in the SHB (5 years per group). A unique feature of these donor heart samples is they enable laboratories to investigate changes in expression of genes and proteins over a period of six decades

collection is probably the single main factor responsible for the high quality of the SHB heart samples.

Non-sarcomeric causes of human heart failure

Figure 2 summarizes papers published by the SHB and its collaborators (Pleuss et al. 2015). Our major contribution was the selection and provision of high-quality failing and non-failing samples of left ventricle. When the research was carried out in laboratories outside of the Sydney, heart samples (usually were left ventricle) were transported to their final destination in a nitrogen dry shipper which maintained temperatures at about -180°C . The dewar shipped maintained temperature for about 2 weeks, which took the pressure of the shippers when deliveries took more than the usual few days.

Signal transduction proteins (STAT and MAPK)

We were the first to report the role of signal transducers and activators of transcription (STATs) and mitogen-activated protein kinases (MAPKs) using LV samples from patients with ischemic heart disease (IHD, 11 samples), dilated cardiomyopathy (DCM, 6 samples), and non-diseased donor LV (4 samples). Significant expression was observed in cardiomyocytes that had a punctate distribution and was not associated with the sarcomeric repeats. MAPKs expression was also post-translationally modified (phosphorylated) only in IHD. Different members of the STAT family were also phosphorylated in both forms of heart failure (Court et al. 2002). In a slightly larger (IHD = 11, DCM = 9, age-matched donors = 9) study of STATs1, 3, 5, and 6, only STAT1 and

STAT5 were phosphorylated in IHD. MAPK p38 was only phosphorylated in IHD. They concluded that only STAT1 and STAT5 are likely to play a role in regulating human heart failure (Ng et al. 2003).

FHOD3

Iskratsch et al. (2010) identified a novel striated muscle-specific splice variant of the formin, FHOD3 that introduces a casein kinase 2 phosphorylation site. The specific targeting of muscle FHOD3 to the cardiac myofibrils is abolished in phosphor-mutants or by the inhibition of CK2. FHOD3 efficiently promotes the polymerization of cardiomyocyte actin filaments and downregulation of its expression severely affects myofibril integrity. In murine and human cardiomyopathy, FHOD3 expression is reduced with a concomitant isoform switch and change of subcellular targeting. The data suggest that a muscle-specific isoform of FHOD3 is required for the maintenance of the contractile structures in heart muscle and that its function is regulated by post-translational modification.

FHOD1

Dwyer et al. characterized the expression and subcellular localization of FHOD3's closest relative, FHOD1, in the heart. Confocal microscopy shows it is mainly located at the intercalated disc, the cell-cell contact between adjacent cardiomyocytes. It is also partially associated with the myofibrils. Subcellular targeting of FHOD1 is probably mediated by its N-terminal domain, since expression constructs lacking

Fig. 2 This figure summarizes the contributions of the SHB to our knowledge of the role of extra-sarcomeric proteins in human heart failure. These proteins are located in the cardiomyocyte cytoplasm, the internal

membranes of the sarcoplasmic reticulum, the sarcolemma of cardiomyocytes including the intercalated disc membranes, the nucleus, and the proteins of the extracellular space

this domain show aberrant localization in primary cultures of neonatal rat cardiomyocytes. Finally, it was shown that in contrast to FHOD3, expression of FHOD1 is increased in DCM LV, suggesting that the two formins play distinct roles and are differentially regulated in cardiomyocytes (Dwyer et al. 2015).

LIM domain proteins

The role of the LIM domain family of proteins and heart failure is an ongoing interest at the SHB (Li et al. 2012). Bovill et al. (2008) first reported that Four-and-a-half LIM-protein 2 (*FHL2*) levels were preserved in non-failing human left ventricle samples, but levels were reduced by 53% in DCM hearts with gross dilatation. *FHL2* was localized in the perinuclear Golgi apparatus complex. This change is associated with a reduction in the levels of several kinases, which might disrupt myocardial energy metabolism in heart failure. Lu et al. (2012) and later Lal et al. (2016) reported that *FHL1* was significantly upregulated in rat models of HCM. Subsequently, Schreckenbach et al. (2013) reported mutations in the *FHL1* gene where four members of a family died of heart failure under 14 years of age. Another LIM domain protein, muscle LIM protein (MLP) is a well-established cause

of human heart failure and is known to cause both HCM and DCM in humans (Bos et al. 2006). It is the loss of MLP from the cytoskeleton that may be the cause (Boateng et al. 2007). More recently, Lange et al. (2016) concluded that the main role of MLP is to inhibit a kinase (PKCa) located at the intercalated disc and that this inhibition leads to heart failure.

Hippo-Yes-associated protein

The transcriptional co-activator Yes-associated protein (YAP) is a key driver of heart growth. It binds to several transcription factors to activate transcription of cell-cycle and cell-survival genes, thus promoting organ growth. YAP and Hippo are essential for normal heart development. YAP activation diminishes with age and Lin et al.'s paper shows that regulation of YAP activity is crucial for normal cardiac growth control. Moreover, they suggest that unknown mechanisms may suppress YAP mitogenic activity in the post-natal heart (Lin et al. 2016).

Coronary artery disease and Hsp27

Robinson et al. (2010) compared non-diseased human coronary arteries with those from IHD to determine the levels of

phosphorylated heat-shock-protein, Hsp27, using Western blots. They reported that its expression is specifically decreased in IHD vessels, but not in non-diseased vessels. Hsp27 is expressed in both smooth muscle and endothelial cells. Two-dimensional difference gel electrophoresis detected proteins that were identified by mass spectrometry. The results are consistent with the hypothesis that phospho-Hsp27 protects against vascular disease possibly by stabilizing the actin cytoskeleton within endothelial and/or smooth muscle cells. In another report, we undertook the identification of basic proteins (pH 6–11) of the human LV using 2-DE. Tissue was lysed and proteins separated on pH 6–11 IPG strips followed by separation on 12% SDS polyacrylamide gels in the second dimension. Proteins were then identified by mass spectrometry and analyzed using in-house analysis. The proteome map contains 176 identified spots with 151 unique proteins and is available on the UCD-2DPAGE database at <http://proteomics-portal.ucd.ie:8082>. This reference map and others will aid future studies of heart diseases such as IDCM and IHD (Polden et al. 2011).

Sarcoplasmic reticulum

Small samples of LV tissue were used to isolate SR vesicles and measure their sensitivity to Ca^{2+} and Mg^{2+} . The performance of the membrane RyR2 Ca^{2+} channels revealed they are sensitive to post-translational modifications including hyperphosphorylation of key serines residues and a reduction in free thiols. These changes did not depend on the nature of the heart failure Walweel et al. 2014, 2017a, b.

P2X receptors, intercalated discs, and sarcolemma

Berry et al. (1998) were the first to identify ATP-specific P2X1 receptors in the human heart. Using Western blots, they noted changes in sub-populations of these channels that were related to the LV ejection fraction of IDCM patients. In 1999, we reported (Berry et al. 1999) Western blots showing increases in the atria (but no significant increase in the ventricles) in DCM samples compared to healthy donor samples. In the next issue of the same journal, Hou et al. (1999) reported that P2X1 mRNA levels were significantly increased in *both atria and ventricles*, and that P2X1 receptors were upregulated in congestive heart failure. However, by the following year (Berry et al. 2000), we had developed more specific antibodies, and, with the increase in the number of SHB hearts, we were compared P2X1 expression (Western blots) in 14 IDCM and 11 healthy donor hearts and observed a significantly increased (~80%) expression in both atria ventricles in DCM patients. The importance of these observations was highlighted in a recent review (Ralevic 2015) who pointed out the possible relevance of these receptors as therapeutic targets.

Changes in the nucleus

Lamins are nuclear protein that came to prominence when it was discovered that mutations in the rod domain of both lamin A and lamin C are a known cause of DCM (Fatkin et al. 1999), but are they homodimers or heterodimers? In 2001, we used Western blots to address this question. We analyzed LV samples from 10 DCM LV samples and non-diseased donors and unexpectedly their evidence led them to conclude the nuclei carried lamin A and lamin C as well as lamin C2, a short lamin associated with the nuclear envelope (Berry et al. 2001). During the hypertrophy of cardiomyocytes, there is an increase in the size and expression of cytoskeletal proteins and a reactivation of a fetal gene program. We investigated the role of a range of nuclear import and export molecules that control of hypertrophy in rat and human heart failure. In cardiomyocytes from patients with DCM, nuclear size was increased and there were changes in nuclear transport molecules related to increased export and decreased import activity. These changes occur early in hypertrophic development, suggesting that hypertrophy can be prevented, or even reversed, by targeting import/export, which may open new therapeutic opportunities (Chahine et al. 2014).

Extracellular matrix proteins

The SHB provided 15 failing (IHD) and 15 non-failing LV to Bill Pu's laboratory at Harvard Medical School to test his hypothesis that the expression and function of long non-coding RNAs (lncRNAs) are differentially regulated in diseased hearts. They performed RNA deep sequencing of protein-coding and non-coding RNAs and found changes in 35 lncRNAs. Expression correlation coefficient analyses revealed a strong association between lncRNAs and extracellular matrix (ECM) protein-coding genes. Overexpressed or knocked-down experiments suggest that lncRNAs are important regulators of fibrosis and the expression of ECM synthesis genes. Thus, lncRNAs may be novel regulators of heart function and cardiac disorders (Huang et al. 2016). Considering the recent observation by David Crossman and his colleagues, these changes in the ECM are consistent with increased collagen around the openings of the T tubules in the sarcolemma, which particularly included type IV collagen (Crossman et al. 2017).

Access to SHB heart samples?

If the above discussion has stimulated readers to attempt using human heart samples to test important hypotheses, they should contact the Sydney Heart Bank via any of the first three authors. We will work with you to design experiments that are statistically relevant, and perhaps avoid a decision to employ an animal model, at least as a first step. You will need (1) a

clear set of aims, (2) a rationale for the amounts of tissue required, and (3) current human ethics approval (or a waiver) for the project. The independent SHB Board will make the final decision and the University of Sydney Research office will arrange for a Material Transfer agreement with the receiving institution. There are no charges for the tissue or the associated clinical data, but the recipient is asked to actual cover the FedEx cost of transporting the nitrogen-vapor dewar to the recipient's laboratory and returning the empty dewar to the SHB. The process typically takes 3–4 months.

Compliance with ethical standards

Conflict of interest C. G. dos Remedios declares that he has no conflicts of interest. A. Li declares that she has no conflicts of interest. S. Lal declares that he has no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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