Journal of Molecular and Cellular Cardiology 48 (2010) 571–573

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Editorial

Phenotyping transgenic animals—An integrated readout of pathophysiology by combining proteomics and metabolomics with cardiovascular imaging

Fuelled by obesity, hypertension, diabetes, and an aging population, heart failure (HF) is increasingly common in the Western population. Its pathophysiology reflects the complex and multifactorial background, and involvement of neurohormones, cytokines and oxidative stress in an interrelated network of pathways and players. Linking gene expression to loss of function in HF has helped to unravel many of these players, such as the central role of tumor necrosis factor-α, and provided successful treatment targets, like the renin–angiotensin system. Moreover, it helped to link the typical (mal)adaptive myocardial phenotypes, such as hypertrophied and atrophied heart, and insulin resistance and cardiac metabolic maladaptation [1]. For instance, several lines of evidence suggest that a diabetic, hyperglycemic and hyperlipidemic environment predisposes to development of myocardial dysfunction, hypertrophy and HF. Initially, a compensatory, nuclear peroxisome proliferator activated receptor (PPAR)-α-mediated adaptive response promotes greater expression of free fatty acids (FFA)-metabolising enzymes, and reliance on FFA as the major fuel, leading to reduced glycolysis, glucose and lactate oxidation, also described as the Randle hypothesis [1]. In the presence of sustained stress, metabolic maladaption is characterised by decreased expression of PPAR-α, downregulation of genes controlling the oxidation of FFA, reactivation of a foetal gene program and increased glucose oxidation [2]. In addition, whilst a healthy heart features oxidation of FFA as predominant source of fuel for contraction in the steady state, it is the metabolic flexibility of switching between various substrates in response to changes in workload that provides the most *hybrid-fuel* efficiency at the given physiological demand. In HF, impaired capacity of FFA oxidation couples with inadequate compensatory increase in glucose oxidation. When the failing heart faces an increased FFA load (as in diabetes or obesity), intramyocardial lipid accumulation occurs. In the absence of significant coronary artery disease, it seems paradoxical that the heart "fails in the midst of plenty" [1]: both FFA and glucose are the main providers of energy for the beating heart. However, energy transfer becomes impaired at the level of intermediary metabolism, phenotypically revealed by a decline in the phosphocreatine [PCr]/adenosine triphosphate [ATP] ratio [3]. [PCr]/[ATP] ratios correlate with the severity of HF and importantly, are a prognostic indicator of mortality in patients. These clinical observations have led to the formulation of the "energy starvation hypothesis" stating that the failing heart is unable to meet the haemodynamic requirements of the body because there is not enough chemical energy available [4]. Given that the myocardial creatine kinase system plays a pivotal role in the buffering and transfer of high energy phosphates from the sites of energy production to the sites of utilisation, this led to the simplistic concept that elevating cardiac creatine levels may prevent the progression of the disease. Creatine, however, is not synthesised in the heart but is taken up by cardiomyocytes against a concentration gradient via the Na^+-creatine cotransporter (creatine transporter, CrT). External creatine supplementation failed to improve the myocardial creatine (and PCr) content due to a downregulation of the CrT in response to high extracellular creatine [5]. Thus, other models were sought for to investigate whether increasing CrT expression would provide a metabolic strategy for treatment of HF [6].

In this issue of the *Journal of Molecular and Cellular Cardiology*, Philips et al. [7] expanded on their previous study [8], reporting that overexpression of the CrT in 24- to 37-week-old mice (CrT-OE mice) led to supranormal myocardial creatine and phosphocreatine concentrations. Unexpectedly, chronically increased myocardial creatine levels in the CrT-OE model were not beneficial, but induced progressive cardiac hypertrophy and HF. The present study now provides elegant links between the cardiometabolic and pathophysiological features of LV remodelling, in a multimodal bio-signature, combing the proteomic/metabolomic readout with cardiovascular imaging in a longitudinal fashion. These investigators observed and contrasted cardiometabolic features of the CrT-OE model with wild-type littermate controls. In this transgenic HF model with overexpression of the CrT biochemical changes, such as the rise in creatine and reduced glycolytic capacity, as evidenced by a reduction in enolase activity and a drop in lactate levels, substantially antedated the onset of detectable left ventricular dysfunction. In addition, the decrease in enolase activity was paralleled by an increase in β-enolase repressor factor, suggesting that the metabolic maladaption is at least partially regulated at the transcriptional level. The indisputable strength of this study is the targeted use of multimodal readouts, based on a variety of very different, but clearly complementary techniques. Magnetic resonance imaging (MRI) provides detection of structural and functional changes in left ventricular remodelling, and a physiological arm of the study [9]. MRI based proton-spectroscopy (H^1-MRS) followed the myocardial creatine content [10], and difference in-gel electrophoresis (DIGE) supplied information on the underlying enzymatic changes. This insightful methodological approach, skillfully joining the 'system biology' with the 'system physiology', is following a similar concept as pursued by our group (Fig. 1): a comprehensive bio-signature of multifaceted cardiovascular disease processes should include the integration of different -omic approaches for the identification (and cross-reference) of the variety of pathophysiological responses, in particular at the protein and metabolite level [11–13].

Studies linking genetic manipulations to phenotypes are not necessarily as unambiguous as sometimes portrayed and clarity is

0022-2828/$ – see front matter
doi:10.1016/j.yjmcc.2009.12.001

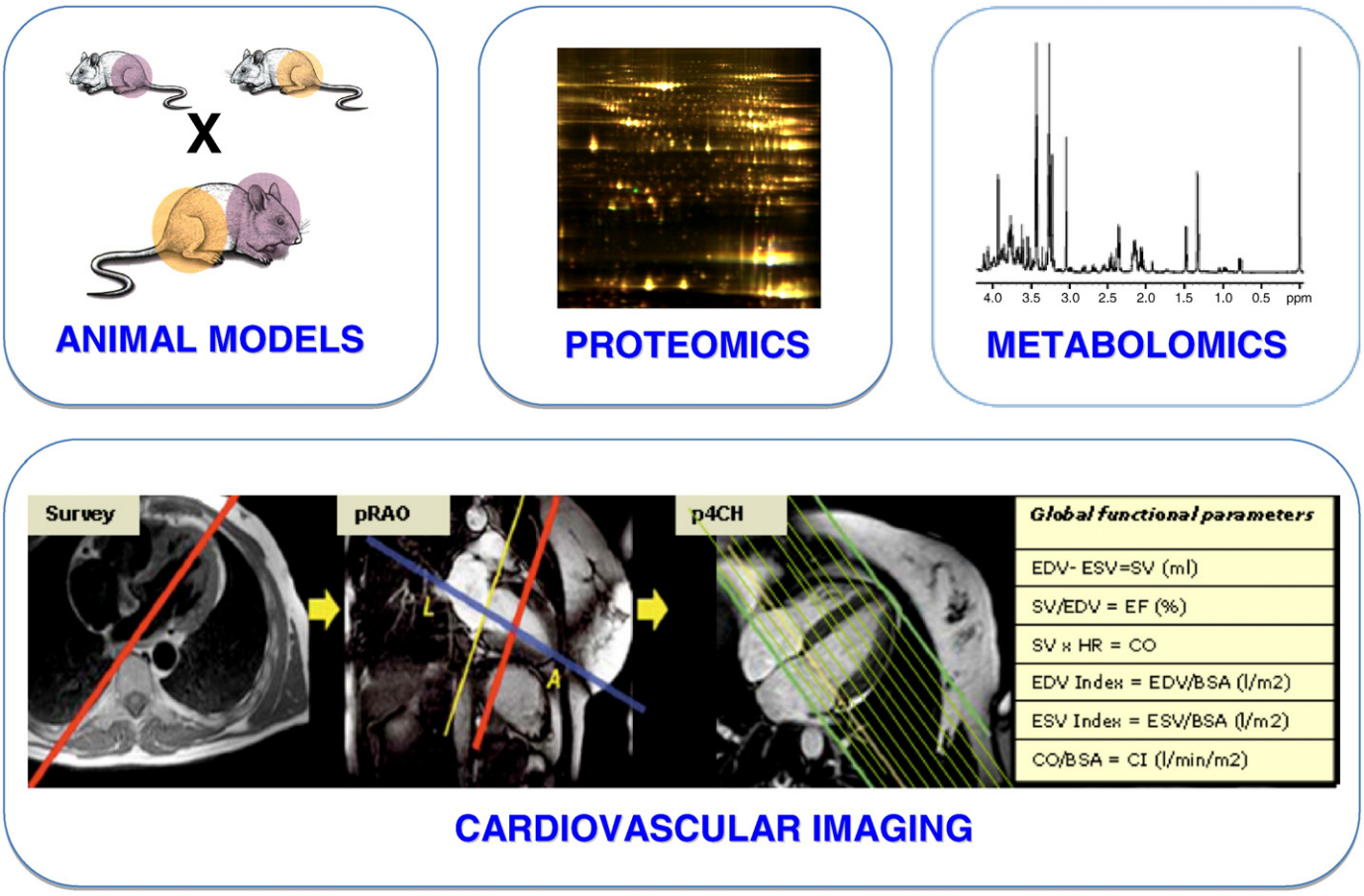

Fig. 1. Comprehensive biosignatures. Phenotyping animal models of cardiovascular disease by combining proteomics and metabolomics with imaging provides an integrated readout of pathophysiology that is essential to further our understanding of network behaviour in response to genetic perturbations. Cardiac MRI allows a comprehensive investigation into cardiovascular physiology. The example provided in the lower panel is a contemporary investigation into cavity volumes and global systolic function using balanced steady-state free precession sequences; following the preparations scans (general survey) and 2 rapid heart axis survey scans (pseudo-2 chamber- and pseudo-4 chamber (CH) views), volumetric cavity assessment is obtained by whole-heart coverage of gapless short-axis (SAX) slices.

not always their consequence [14]. Transgenic animals undeniably play a central role in revealing potential mechanisms that operate *in vivo*. Whether these models appropriately substitute for the 'descriptive and holistic approach' of model systems and whether the experimental nature of inducing disease-like syndromes in non-human species allows a straightforward translation onto the human phenotype, remains hotly debated [14,15]. Clearly, a genetic manipulation leads to a significant misbalance in the biological network of interrelated pathways as a whole: rather than resulting in a single gene-molecule effect, the readouts reveal complex changes. Yet, there is still a general lack of control for compensatory off-target effects. Instead, the phenotype is directly attributed to the primary genetic perturbation. In combination with a systems biology approach, transgenic animal models could reveal more profound insights into disease mechanisms.

The use of CrT-OE mice in the present study of cardiac metabolism by H^1-MRS illustrates this point. Whilst H^1-MRSenables the detection of total creatine, it is the ^{31}P-MRS that allows detection of cardiac energetic metabolites, such as PCr, ATP, inorganic phosphate and intracellular pH [16]. Transgenic models of low creatine (guanidinoacetate-*N*-methyltransferase-knockout mice, GAMT−/−) [17] and high cardiac creatine levels (CrT-OE mice) [8] both produce HF-like phenotypes, with left ventricular dysfunction and remodelling, and cardiometabolic impairment. Yet, they produce differential [PCr]/[ATP] MRS readouts; whilst the former clearly features reduced activity of these metabolites, in CrT-OE mice, the levels of these two metabolites are increased. In CrT-OE mice, the metabolic impairment stems from increased free ADP concentrations, which may in turn, result in a reduction in the available free energy of ATP hydrolysis, and potentially directly contribute to contractile dysfunction. Thus, the use of MRS in this transgenic model may have potentially 'phenotyped' homeostatic perturbation related to this particular transgenic mouse, and contributed less to a better understanding into the role of energy metabolism in human HF [15]. Nonetheless, if increased free creatine results in decreased glycolytic capacity, this is a truly novel mechanism. Future studies are required to establish the time course of PCr/total creatine abnormalities in order to define the relation between left ventricular dysfunction and creatine metabolism. Similarly, isolated perturbation of enolases, but not other glycolytic enzymes is not a strong argument for impaired glycolysis in the present context of HF. Alternative explanations for the modest drop in lactate are more lactate efflux from the heart or less lactate production, i.e. the flux through the glycolytic pathway is similar but more pyruvate is oxidised in mitochondria.

The advent of -omics technologies increases our recognition that genetic manipulations frequently lead to alterations of seemingly unrelated biochemical pathways. The study of Phillips et al. highlights the importance of analysing compensatory changes in transgenic animal models, in particular the effects on metabolism [18], before interpreting cardiovascular phenotypes, and demonstrates that such a comprehensive approach can reveal new aspects of cardiovascular pathophysiology with potential therapeutic implications.

Acknowledgements

Dr. M. Mayr is a Senior Research Fellow of the British Heart Foundation.

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20 November 2009