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T1 ENERGETIC DEFICIENCY AND ADENOSINE RECEPTOR SIGNALLING IN CARDIAC FIBROSIS

[1]A Stockenhuber, [1]S Ghafari, [1]M Kelly, [1]V Steeples, [1]G Czibik, [2]D Tyler, [3]N Støttrup, [1]A Yavari, [1]H Watkins, [1]H Ashrafian. [1]*Radcliffe Department of Medicine, University of Oxford, Oxford, UK*; [2]*Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK*; [3]*Department of Clinical Medicine, Aarhus University*

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Myocardial fibrosis (MF) contributes to the pathogenesis of cardiac hypertrophy secondary to energetic perturbation. Recent evidence suggests an increase of purine signalling upon energetic deficiency and specifically a role of adenosine signalling in tissue fibrosis. The specific objective of this study was to delineate adenosine A2A receptors in the development of MF.

In vitro, isolated cardiac fibroblasts demonstrated a significant increase in collagen production upon A2A receptor stimulation. This was inhibited by addition of a specific A2A receptor inhibitor.

In vivo, models of cardiac hypertrophy including the transverse aortic constriction (TAC) model and cardiac actin E99K transgenic mice (E99K mice) were investigated as murine models for MF.

In E99K mice a reduced phosphocreatine/ATP ratio was demonstrated using magnetic resonance spectroscopy. Interstitial adenosine levels measured by microdialysis correlated with collagen content, showing energy deficiency and a correlation with MF. Crossbreeding of E99K and Adenosine A2A receptor knock out (A2A KO) mice resulted in a significant reduction of MF in E99K heterozygous A2A KO animals.

A2A KO mice undergoing TAC demonstrated significantly less fibrosis formation compared to wild type mice upon measurement of myocardial collagen and on histology. This was associated with a significant rescue of cardiac function.

Finally, pharmacologic adenosine A2A receptor inhibition using the antagonist ZM241385 demonstrated a partial rescue effect on MF in both TAC and E99K animals.

This data indicates that signalling of energy deficiency via adenosine A2A receptors play a crucial role in the formation of MF and that this pathway is susceptible to pharmacologic modulation.

T2 FACTOR INHIBITING HIF (FIH1) MODULATES CARDIAC FUNCTION AND METABOLISM

[1]J Holliday, [1]S Platt, [1]MK Handzlik, [1]IA MacDonald, [2]K Clarke, [3]PJ Ratcliffe, [1]MA Cole. [1]*School of Life Sciences, University of Nottingham*; [2]*Department of Physiology, Anatomy and Genetics University of Oxford*; [3]*Henry Wellcome Building for Molecular Physiology, University of Oxford*

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Hypoxia-inducible factor (HIF) plays a pivotal role in the cellular response to reduced oxygen availability. HIF activity is regulated by two families of oxygen sensitive enzymes; the prolyl hydroxylase domain (PHD) family, and factor-inhibiting HIF (FIH1). FIH1 is thought to be an essential regulator of metabolism but its role in the heart is unknown.

Mice with a null mutation in the FIH1 gene (FIH1-/-, $n \geq 5$) had 18% lower body weight ($p<0.02$) than wild type littermate controls (WT, $n \geq 6$), but normal total cardiac mass. Right ventricular mass determined via MRI was 25% greater in FIH1-/- hearts ($p<0.01$). Cine MRI revealed a 15% reduction in stroke volume in FIH1-/- hearts, from 27.4 ± 2.3 µl in WT to 23.4 ± 1.5 µl in FIH1-/- ($p<0.05$). Impaired contractility was also observed in individual myocytes (sarcomere shortening was $3.01\% \pm 0.20\%$ in FIH1-/- compared to $3.92\% \pm 0.17\%$ in WT, $p<0.05$) and was associated with reduced Ca2+ transient amplitude (fura-2 ratio 0.21 ± 0.02 and 0.29 ± 0.02 for FIH1-/- and WT respectively, $p<0.05$).

Glycolytic flux (µmol/min/g) was significantly higher in Langendorff perfused FIH1-/- hearts (1.17 ± 0.04) than WT (0.79 ± 0.12, $p<0.05$) although no changes in lactate efflux were detected. There were no differences in pyruvate dehydrogenase kinase 1 and 4 protein expression and citrate synthase activity (µmol/min/mg) was similar for both WT (1.01 ± 0.02) and FIH1-/- (0.96 ± 0.03) hearts.

Our data suggest a novel role for FIH1 in modulating cardiac contractility and metabolism, with FIH1 ablation producing cardiac effects comparable to those associated with activation of the hypoxic signalling pathway.

T3 CARDIAC ARRHYTHMIA RESULTING FROM AN ACCUMULATION OF BRANCHED CHAIN AMINO ACIDS IN A MOUSE LINE WITH A MUTATION IN BCAT2

[1]T Nicol, [2]S Podliesna, [2]V Portero, [1]S Falcone, [1]A Blease, [2]S Casini, [2]CR Bezzina, [2]CA Remme, [1]P Potter. [1]*Disease Model Discovery Group, Mammalian Genetics Unit, MRC Harwell Institute, Oxfordshire, UK*; [2]*Department of Experimental Cardiology, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands*

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As part of a large-scale phenotype-driven screen we identified a line exhibiting sudden death. Mapping and whole genome sequencing identified a missense mutation in the Bcat2 gene, encoding mitochondrial branched chained aminotransferase, resulting in an early stop (Q300*) and a truncated protein. Homozygous mice exhibited increased plasma and urine levels of branched chain amino acids (BCAAs). Mutations in this pathway have previously been associated with Maple Syrup Urine Disease (MSUD) and can result in neurological symptoms. All homozygous mice died suddenly at 7 weeks of age, without any preceding symptoms. No cardiac abnormalities were observed on histological analysis, nor were there any other significant findings related to MSUD. An accumulation of branched chain amino acids was identified in urine and serum from homozygous mice, but unlike MSUD there was no accumulation of branched chain keto-acids. Homozygous mice showed QTc-prolongation *in vivo* on surface ECG analysis and prolonged action potential duration (APD) *ex vivo* (assessed by optical mapping in isolated hearts). Moreover, isolated hearts from mutant animals displayed increased inducibility of atrial and ventricular arrhythmias. In line with this, patch clamp measurements revealed significant APD90