Abstracts

P11 PLASMA MEMBRANE CALCIUM ATPASE 1 GENE EXPRESSION INCREASES IN VASCULAR SMOOTH MUSCLE CELLS TREATED WITH INDUCERS OF PULMONARY ARTERIAL HYPERTENSION

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Pulmonary arterial hypertension (PAH) is a chronic and life-threatening disease characterised by a progressive narrowing and occlusion of small pulmonary arteries leading to increased pulmonary resistance, right ventricular hypertrophy, and, finally, right ventricular failure.

Several studies have demonstrated that proliferation and migration of pulmonary arterial smooth muscle cells (PASMCs) play a pivotal role in the vascular remodelling characteristic of PAH. Levels of cytoplasmic calcium are an important determinant of PASMC proliferation and migration. The Plasma Membrane Calcium ATPase (PMCA) proteins extrude calcium from the cytosol to the extracellular medium.

Here, we have investigated whether inducers of PAH trigger any changes in the expression of PMCA genes in PASMCs. Treatment of PASMCs with the PAH inducers Platelet Derived Growth Factor (PDGF) or TNF-alpha induced a significant increase in the RNA levels of PMCA1. PMCA1 RNA levels are also elevated in lungs of rats with monocrotaline-induced PAH. In silico analysis of the PMCA1 gene promoter region has shown putative binding sites for the transcription factors NFAT and NFkB that could mediate the transcriptional upregulation triggered by PDGF and TNF-alpha respectively. However, we show here that upregulated PMCA1 gene expression is not mediated by binding of these transcription factors to the proximal promoter region. No changes were observed in the RNA levels of PMCA4, the other major PMCA isoform expressed in PASMCs.

Our results suggest an important role for PMCA1 in PASMC deregulation during PAH, although a full understanding of the role of PMCA1 on the onset and progression of PAH requires further investigation.

P12 A NOVEL MODEL OF CARDIOMYOPATHY REVEALS A TISSUE SPECIFIC ROLE FOR THE COMPLEX I ASSEMBLY FACTOR ECSIT

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Here we present a mouse model with a missense mutation in the gene Ecsit that shows a progressive cardiomyopathy from 4 weeks of age with no other overt phenotypes. ECSIT is known to play a role in development and immune signalling but is also thought to function as an assembly factor of complex I.

Western blot analysis of tissue lysates revealed a significant reduction in complex I proteins in heart tissue, whereas all other complexes were unaffected. In addition, Seahorse analysis of isolated mitochondria shows a significant reduction in the respiration rates of cardiac mitochondria, whilst no differences could be seen in mitochondria isolated from brain tissue.

In-gel activity demonstrated a significant drop in complex I activity of cardiac mitochondria, whilst brain mitochondria are maintained at close to normal levels. Blue native PAGE performed on cardiac mitochondria shows that this mutation affects ECSIT’s role in a limited number of complex I sub-assemblies. However, this is unique to the heart and mitochondria from brain tissue show no changes in any of the same sub-assemblies, supporting the initial findings that there is normal complex I assembly in the brain.

A potential mechanism lies in the discovery of a previously undescribed 16 kDa fragment of ECSIT that is present in WT cardiac mitochondria but not in mutant. This fragment is also undetectable in mitochondria isolated from brain tissue, indicating a tissue specific cleavage of ECSIT protein as a method of action.

P13 CHANGES IN MITOCHONDRIAL MORPHOLOGY & DISTRIBUTION DURING POSTNATAL DEVELOPMENT

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Vulnerability to cardiac ischemia/reperfusion injury (IRI) changes during postnatal development, with greatest resistance at postnatal day 14 (P14) in rats. The underlying mechanism is currently unknown. We hypothesise that developmental changes in mitochondrial morphology and distribution of the 3 mitochondrial subpopulations – interfibrillar, perinuclear and subsarcolemmal – may be linked to these differences in vulnerability.

A potential mechanism lies in the discovery of a previously undescribed 16 kDa fragment of ECSIT that is present in WT cardiac mitochondria but not in mutant. This fragment is also undetectable in mitochondria isolated from brain tissue, indicating a tissue specific cleavage of ECSIT protein as a method of action.

Hearts from P14 (n=3) and adult (n=3) rats were perfused with Krebs-solution, followed by fixative solution (1% glutaraldehyde, 1% paraformaldehyde), before processing and embedding in EPON epoxy resin for electron microscopy. Images were taken using a Tecnai12 TEM electron microscope, from which areas, widths, lengths and densities of mitochondria from the 3 subpopulations were measured using ImageJ.

All 3 mitochondrial subpopulations had statistically significantly larger mean area, lower aspect ratio, and greater roundness in adult compared with P14 hearts. Adult hearts had a significantly higher density of interfibrillar but lower density of perinuclear mitochondria than P14 hearts. There were also differences in subpopulations within each age group. At P14, interfibrillar mitochondria had a greater area but lower roundness in comparison with perinuclear and subsarcolemmal mitochondria, the two of which showed no significant difference from one another. In adults however, only interfibrillar and subsarcolemmal mitochondria showed significant differences in area, whereas all 3 subpopulations differed in roundness.

These data demonstrate marked differences in mitochondrial morphology and distribution between P14 and adult...
hearts. Whether these distinct characteristics are altered differently by an ischaemic insult, thus explaining variations in vulnerability, is not presently known. This is currently under investigation.

P14 MITOCHONDRIAL REGULATION OF EXOSOMAL HYPERPOLARIZED 13C MICRONA CARGO MEDIATES CELL PROLIFERATION IN SYNTHETIC VASCULAR SMOOTH MUSCLE CELLS

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In this work we aimed to study potential correlation between the synthetic hyperproliferative vascular smooth muscle (VSM) cell and exosomal-dependent signalling.

We have previously shown mitochondrial bioenergetics are upregulated in synthetic hyperproliferative VSM cells.

Total exosomal release was increased in synthetic VSM cells vs wild type (WT) cells. Total protein and RNA within exosomes in synthetic VSM was significantly greater when compared with WT VSM cells. Addition of exosomes isolated from WT or synthetic hyperproliferative to VSM cell cultures resulted in 4.7±2.3% and 23.4±4.6% respectively, p<0.05. qRT-PCR of exosomal contents (synthetic VSM cell vs WT VSM cells) highlighted a significant reduction in pro-apoptotic genes (Bnip3, SOD1, SOD2), a reduction in significant tumour suppressor genes (Pmaip1, p53) and significant reduction in cell cycle regulator Cdkn2a. Likewise, RNA for P13K, 4EBP1 and mTOR were all significantly greater in exosomes isolated from synthetic VSM cell vs WT VSM cells. RNAseq sequencing highlighted significant differences in exosome contents. Notably a 10-fold increase in pro-mitogenic/synthetic miR21 and 3.5-fold loss of anti-mitogenic/synthetic miR145, synthetic VSM cell vs WT VSM cells. The use of selective anti-miR/miR mimetics in cell proliferation assays confirmed that both miR145/miR21 are regulators of VSM cell phenotype and proliferation.

Inhibition of mitochondrial bioenergetics or mitochondrial dynamics restored the exosomal yield, exosomal contents, RNA and miRNA similar to that measured in WT cells.

Our results implicates exosomes and their miRNA contents as crucial mediators of cellular proliferation in synthetic VSM cells

P15 CYCLOSPORINE A IS PROTECTIVE AGAINST OXIDATIVE STRESS IN ADULT BUT NOT IN IMMATURE ISOLATED CARDIOMYOCYTES

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The vulnerability of intact rat hearts to ischaemia and reperfusion injury changes during post-natal development with 14 days old being most resistant. The mechanism for this is multifactorial, but one significant element is the changing characteristics of the mitochondrial permeability transition pore (MPTP). We investigated the effect of oxidative stress and Ca2+ loading on cardiomyocyte viability in cell suspension through development with or without MPTP inhibition.

Ventricular cardiomyocytes were isolated enzymatically from 14 (n=4), 28 day old (n=3) and adult rat heart (n=3). These cardiomyocytes were incubated with 0.5 mM H2O2 and 3 mM Ca2+ (simulated reperfusion) in the presence or absence of 2 μM Cyclosporine A (CsA). Cardiomyocytes incubated in normal buffer were used as control. Cardiomyocyte viability (assessed using trypan blue) and morphology was monitored every 30 min for 2 hours.

There was a time-dependent decrease in viability in all age groups. However, this effect was more marked in 14 day old compared to adult and 28 day old cardiomyocytes. In the adult group, CsA significantly improved cardiomyocyte viability at all time points (at 120 min, 76%±7.4 vs 53%±5.3). No protective effect was seen in 14 (22%±12.7 for CsA vs 10%±6.6 injured control) or 28 day old cardiomyocytes (55%±7.9 for CsA vs 33±5.4 for injured control).

Unlike intact adult heart, the resistance of isolated cardiomyocytes to simulated reperfusion injury increases from 14 days of age to adulthood. The finding that CsA does not confer protection in the younger age group could be due to excessive injury or due to changes in MPTP sensitivity.

P16 HYPERPOLARIZED 13C MAGNETIC RESONANCE SPECTROSCOPY IDENTIFIES CHANGES TO MYOCARDIAL METABOLIC FLUXES IN A RAT MODEL OF DOXORUBICIN-INDUCED CARDIOTOXICITY

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Doxorubicin (DOX) is a widely used anthracycline chemotherapeutic for adult and paediatric patients and its use has greatly improved cancer survival rates. However, DOX can cause serious cardiac side effects leading to congestive heart failure. The mechanisms for this toxicity are not yet fully understood, although mitochondrial oxidative stress and altered cardiac energetics are thought to play a key role in the pathology. In this study, we measured real-time metabolic fluxes in the rat heart following DOX treatment using hyperpolarized 13C magnetic resonance spectroscopy (MRS).

Rats were treated i.v. weekly for 6 weeks with either 2 mg/ kg DOX (n=12, low-dose) or saline (n=12), or for 5 weeks with 3 mg/kg DOX (n=8, high-dose) or saline (n=8). CINE MR imaging for cardiac functional analysis and hyperpolarized [1–13C]- and [2–13C]pyruvate MRS were performed at weeks 1, 3 and 6.

DOX treatment lead to a progressive and dose-dependent decrease in cardiac ejection fraction and cardiac output. Those functional changes were accompanied by reduced pyruvate dehydrogenase flux in the high-dose model and reduced 13C label incorporation into the glutamate and acetyl-carnitine pool in both models, suggesting altered citric acid cycle flux and reduced acetyl-CoA buffering capacity in the myocardium. Rats showed variability in cardiotoxic severity and the metabolic and functional changes were significantly correlated.

Hyperpolarized 13C MRS is therefore a unique non-invasive method to reveal early metabolic effects of DOX on the heart. Future research will focus on unravelling the