Our findings identify ALDH2 as a critical metabolic checkpoint during endothelial branching behaviour.

Methods: Human umbilical vein endothelial cells were cultured until senescence in the presence or absence of ALDH2 inhibitor, daidzin at doses of 10 μM. Senescent cells were compared with non-senescent cells. Cell proliferation, sprouting and miRNAs expressions were studied.

Results: Pharmacological inhibition of ALDH-2 in EC induces a marked upregulation of senescent markers, such as ALDH1A3, p16, and p21 that interfere with sprouting capability and proliferation in vitro assays. Further, senescent EC show a reduced cumulative population doubling, decreased telomerase activity. We find that ALDH2 regulates miRNAs involved in cell senescence and reduces signalling by pro-angiogenic factors, including VEGF. Modulation of miRNAs by mimic or antagomir in ALDH2-expressing endothelium normalizes EC growth and senescence.

Conclusion: Our findings identify ALDH2 as a critical metabolic checkpoint during endothelial growth and senescence.

Background: Heart failure (HF) is a cardiovascular syndrome with high morbidity and mortality. Among the mechanisms involved in the etiopathogenesis of the failing heart, monoamine oxidase (MAO) is emerging as a major ROS source with potential pathophysiological relevance. MAO is a flavin-dependent enzyme involved in the catabolism of biogenic amines and related precursors. Using several biochemical, metabolic and biological tools, we conclude that alteration of the N- or C-terminus of the G-PCR should not affect its mechanosensitivity as the identified residues were distributed throughout the trans-membrane helices.

Methods and Results: We have built the world's first mechanosensor assay, borrowing techniques from synthetic biology. Cells are sensitive to mechanical forces, but little work has been done to develop assays to monitor mechanosensor activity. Furthermore, it is currently impossible to use mechanosen- sor activity to drive gene expression.

Results: A novel assay for regulating transcription factors by flow

Conclusions: We have built the world’s first mechanosensor assay, borrowing techniques from synthetic biology, which is capable of regulating transcription factors in a flow dependent manner.

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Remote ischaemic conditioning reduces infarct size in animal in vivo models of ischaemia-reperfusion injury: a systematic review and meta-analysis

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Background: The efficacy of remote ischaemic conditioning (RIC) at ameliorating myocardial ischaemia-reperfusion injury (IRI) in patients has been called into question by recent neutral large randomized controlled trials.

Purpose: We aimed to analyse the pre-clinical evidence base to ascertain the overall effect of RIC in animal in vivo models of myocardial IRI. Furthermore, we aimed to investigate whether specific experimental variables affect the protective utility of RIC in animal models and might explain the difficulty in translating this promising therapeutic strategy.

Methods: Our primary outcome measure was the weighted mean difference in infarct size between RIC and control groups in in vivo models of myocardial IRI, which we pooled using random-effects meta-analysis. Subgroup analyses were performed using univariate meta-regressions to explore which experimental factors and quality indicators contribute to heterogeneity.

Results: A systematic review returned 34 reports, from which we made 48 controlled comparisons of RIC vs a pooled random-effects meta-analysis. In total, our analysis includes data from 305 control animals and 418 animals subject to RIC. Overall, RIC reduced infarct size as a percentage of area at risk by 20.9% (95% CI 18.2–23.7), when compared to untreated controls (P < 0.001; see Figure 1). Interestingly, we observed significant heterogeneity in effect size (T2 = 92.9 and I2 = 99.4%; P < 0.001), however, this could not be explained by any of the experimental variables analysed by meta-regression.

Conclusions: RIC significantly reduced infarct size in in vivo models of myocardial IRI. Significant heterogeneity between studies could not be explained by experimental variables, which may implicate co-morbidities or other medications and techniques as the cause of disconnect between laboratory and human studies. Importantly, our findings suggest that in the context of in vivo studies to date the optimal RIC stimulus has not yet been identified.

The efficacy of remote ischaemic conditioning (RIC) at ameliorating myocardial ischaemia-reperfusion injury (IRI) could not be explained by experimental variables, which may implicate co-morbidities or other medications and techniques as the cause of disconnect between laboratory and human studies.

664
A proteomic investigation into the mechanisms of VEGF-adhesion receptor crosstalk in endothelial cells

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Introduction: Abnormal angiogenesis contributes to the development of diseases, and intervening in the process presents a promising therapeutic strategy. Vascular endothelial growth factor (VEGF) plays a key role in regulating angiogenesis, in part through co-ordination with endothelial cell integrin adhesion complexes (IACs), structures that form upon cell adhesion receptor interaction with extracellular matrix ligands such as fibronectin (FN).

Purpose: This project aims to identify mediators of VEGF-adhesion receptor crosstalk.

Methods: An IAC enrichment protocol for human umbilical vein endothelial cells (HUVECs) was established. Briefly, HUVECs were plated on FN for 2 hours to allow IAC formation, and treated with serum-free medium or VEGF (25 ng/ml) for 10 minutes. HUVECs were immediately crossedlinked to stabilise IACs, followed by cell body removal, and collection of remaining IACs for analysis.

Results: Mass spectrometry (MS) analyses of IACs enriched from HUVECs defined a FN-mediated network of 401 proteins (identified with >3 unique peptides). This dataset compares favourably to previously reported IAC composition datasets, confirming successful identification of IAC proteins using this system. In addition, western blotting and immunofluorescence validated a selection of proteins identified. Subsequent MS analyses revealed that the abundance of only 3.5% of HUVEC IAC proteins changed greater than two-fold after VEGF treatment.

Conclusions: We propose that whilst IAC composition remains largely unchanged, kinase activity within IACs may regulate adhesion signalling during VEGF-adhesion crosstalk. To investigate this hypothesis, a phosphoproteomic workflow has been developed to analyse HUVEC IAC phosphopeptides. Proteomic and phosphoproteomic datasets will be produced and used to generate hypotheses for the mechanisms by which endothelial cell IACs co-ordinate their activity with VEGF signalling, and will be tested using suitable models of angiogenesis.

665
Assessing the role of PMCA1 in arrhythmia development relating to β-adrenergic signalling

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Background/Introduction: Over 2 million people each year in the UK experience an arrhythmic event and many sufferers have an underlying genetic determinant. The sympathetic nervous system, working through β-adrenergic signalling, is a known regulator of arrhythmogenesis, with β-blockers used in the management of arrhythmias.

Purpose: Here we aim to identify a new role for a gene linked to several features of heart failure, Atp2b1 (Plasma membrane calcium ATPase 1, PMCA1). With the pump previously being associated with sympathetic signalling, we believe PMCA1 may influence heart rhythm stability in relation to β-adrenergic stimulation.

Conclusion: Our data strongly suggest that Gαq is important in defining the correct transcription program of cardiomyocytes and in gene expression re-programming during cardiac hypertrophy, regulating the expression of genes involved in heart homeostasis and cardiac hypertrophy. This project may lead to the development of new therapeutic strategies for HF based on the modulation of this epigenetic enzyme.
Methods: To assess the PMCA1 in heart rhythm control associated with sympathetic stimulation, cardiomyocyte-specific knockout mice (PMCA1CKO) and controls were treated with the \(\beta\)-adrenergic agonist isoprenaline (10mg/kg/day) for 7 day period via subcutaneous mini-osmotic pumps. Before and during the course of treatment heart rhythm stability was monitored using in vivo electrocardiography. Cardiac function was also assessed at day 7 by haemodynamic analysis.

Results: Pre-treatment, PMCA1CKO mice displayed abnormal heart rhythms related to cardiac repolarisation dysfunction. This was evident by prolonged QTc and JT intervals (\(p<0.01\)). Following isoprenaline treatment, QTc and JT intervals appeared to be restored to a level comparable with controls. These changes were evident at both 2 days and 7 days post-treatment. Additionally, PMCA1CKO mice had normal cardiac expression of several sympathetic signalling components, including \(\beta\)-adrenergic receptors.

Conclusion: Our findings suggest PMCA1 influences heart rhythm, with reduced expression resulting in cardiac repolarisation dysfunction. Furthermore these changes appear to be occurring through a pathway independent to \(\beta\)-adrenergic signalling. Translationaly, these findings add to the emerging evidence re-evaluating the use of \(\beta\)-blockers in some cohorts of arrhythmia patients.

Abstracts

667

The expression of beta myosin isoform MYH7B correlates with severity of left ventricular systolic dysfunction in patients with hypertrophic cardiomyopathy

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Background: Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiomyopathy and occurs in 1 out of 500 newborns. Approximately 15-20% of HCM patients develop left ventricular dysfunction (LVD) and can die prematurely due to heart failure (HF). A shift from the faster alpha-myosin heavy chain (MHC) (MYH6) towards the slower fetal beta-MHC (MYH7 and its isoform MYH7B) seems to be involved in the onset and progression of HF in HCM. Nevertheless, the underlying mechanisms of this phenomenon are incompletely understood.

Purpose: Aim of the current study was to investigate the relationship between clinical and functional data and the molecular mechanisms involved in myosin shift in HCM patients undergoing myectomy. We have also investigated the role of micro-RNA (miRNA) involved in the epigenetic control of MYH6, MYH7 and MYH7B expression.

Methods: Before surgery, patients underwent clinical evaluation, electrocardiography, echocardiography and cardiac magnetic resonance (CMR) and a blood sample was withdrawn. Global left ventricular function was assessed by measuring percent ejection fraction (EF%) with CMR. Myocardial tissue and plasma were used for the analysis of a panel of selected genes and miRNAs related to myosin shift phenomenon by Taqman assay.

Results: Fourteen patients were enrolled (57 ± 10 years; six out of 14 patients presented with advanced heart failure (NYHA Class III-IV). LVD inversely correlated with MYH7B gene expression (figure A). The level of expression of miRNA-499, which is encoded by MYH7B, inversely correlated with septal wall thickness (figure B). In addition, plasma levels of miRNA-499 positively correlated with MYH7B protein expression (R²=0.6, p=0.0038).

Conclusion: In HCM, higher MYH7B expression was associated with worsening of systolic function. miRNA-499 could become a potential circulating biomarker of myosin shift phenomenon and progression from LVD to failure.

668

Spatial heterogeneity of sympathetic response in the rabbit myocardium

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Introduction: The heterogeneous nature of sympathetic innervation in the myocardium may generate a vulnerable substrate for ventricular arrhythmias in normal and pathological conditions. Examining innervation-related heterogeneity in isolated hearts is possible with pharmacological activation of sympathetic nerves.

Purpose: To determine whether Tyramine, a sympathomimetic amine, can reveal gradients of \(\beta\)-adrenergic nerve response in the normal rabbit ventricle using voltage sensitive dyes.

Methods: Hearts (n = 7) from male New Zealand white rabbits were perfused by Langendorff method. Intrinsic rhythm was controlled by AV node ablation using formalin injection and hearts were paced at 3 Hertz. Hearts were perfused with 10 \(\mu\)M blebbistatin and loaded with Di-4-AN-NEPPS. Optical action potentials were measured at baseline and after 5 min perfusion of Tyramine (10 \(\mu\)M). A washout period was included before 5 min perfusion of Isoprenaline (100nM).

Results: Isoprenaline caused a significant reduction in overall epicardial left ventricle (LV) action potential duration at 50% repolarisation (APD50) (Relative APD50 = 92 ± 1% P < 0.001). Tyramine showed comparable inotropic and chronotropic responses (data not shown) with a similarly significant reduction in overall epicardial APD50 (Relative APD50 = 92 ± 1% P < 0.003). However, with Tyramine, there was a significant shortening in APD50 at the base (APD50 = 89 ± 1% P < 0.01) of the LV compared to the apex (APD50 = 93 ± 1% P < 0.01). Whilst Isoprenaline showed no significantly different response across the basal, medial or apical epicardial surfaces of the LV

Conclusion: Tyramine induced activation of sympathetic nerves revealed a gradient of sympathetic response in the LV, predominantly on the basal to apical axis consistent with known patterns of sympathetic innervation. Therefore, Tyramine may be used to examine the regional basis of dispersion of repolarisation associated with the precipitation of ventricular arrhythmogenesis.