lipid-lowering treatments. Coronary artery bypass grafting (CABG) is commonly used to bypass coronary arteries diseased by atherosclerosis, routinely using the saphenous vein (SV) as a conduit. Early after grafting the SV adapts to the arterial environment through re-endothelialisation, and increased motility of smooth muscle cells (SMC). A clinical association between Lp(a) and coronary artery disease is evident; however, its role in vein graft failure is less clear. Endothelial cells (EC) and SMC were cultured from the SV of patients undergoing CABG. The influence of apo(a) on cellular activity was examined by proliferation (cell counting), chemotaxis (modified Boyden chamber) and chemokinesis (scratch wound) assays. Apo(a) significantly inhibited SV-EC proliferation ($n=9$, $p<0.001$). Although no effect on SV-SMC proliferation was apparent, apo(a) markedly modulated SMC motility and appeared to act as a chemorepellent. When SMC were acutely exposed to a gradient of apo(a), they consistently migrated away from the source ($n=6$, $p<0.01$). Chronic exposure to apo(a) in the scratch wound model also revealed that the speed of migration was reduced ($n=5$, $p<0.01$). Remodelling of the SV is essential for adaptation to an arterial environment, and key to its function as a successful bypass graft. Our studies show that apo(a) inhibits EC proliferation, potentially compromising endothelial repair in the grafted vein. Furthermore, the chemorepellent effect of apo(a) may also impede critical SMC migration required for effective integration. Lp(a) is therefore likely to contribute to impaired SV adaptation and inferior graft patency.

BAS/BSCR30 CRUCIAL ROLES OF CBX3 IDENTIFIED BY NUCLEAR PROTEOMICS IN SMOOTH MUSCLE DIFFERENTIATION FROM STEM CELLS AND VASCULAR INJURY-INDUCED NEOINTIMA FORMATION

doi:10.1136/hrt.2010.205781.41

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Rationale Our previous studies have developed an efficiency method for producing a large number of smooth muscle cells (SMCs) from embryonic stem (ES) cells. However, little is known about the underlying mechanism.

Methodology and results Nuclear proteins were harvested and isolated from undifferentiated and differentiating ES cells at different time points, and subjected to proteomics analysis. Notably, the majority of upregulated nuclear proteins during SMC differentiation were involved in chromatin remodelling, cellular morphogenesis, cell proliferation, DNA replication, protein synthesis, mRNA transport and RNA processing processes. We further focused on chromobox protein homologue 3 (Cbx3) owing to its involvement in the regulation of gene-specific expression. Knockdown of Cbx3 in the differentiating ES cells resulted in downregulation of smooth muscle differentiation markers, while enforced expression of this gene enhanced SMC differentiation in a dose-dependent manner. Our data also suggested that Cbx3 mediates SMC differentiation from ES cells through regulation of smooth muscle-specific transcription factor, serum response factor (SRF) and its coactivator myocardin. Furthermore, we also demonstrated that another smooth muscle transcription factor, Dia1, functions as bridge protein between Cbx3 and SRF, through which Cbx3 modulates SRF activation, and mediates ultimately SMC differentiation from stem cells. Importantly, in vivo perivascular knockdown of Cbx3 significantly increased wire-injury-induced neointima formation in mice.

Conclusions Our findings demonstrated for the first time that Cbx3 has a crucial role in SMC differentiation and possesses an important protective function in vessel injury-induced neointima formation, indicating that Cbx3 could be a potential new therapeutic target for intervention in SMC proliferative-related vascular diseases.

BAS/BSCR31 METABOLIC HOMOEOSTASIS IS MAINTAINED IN MYOCARDIAL HIBERNATION BY ADAPTIVE CHANGES IN THE TRANSCRIPTOME AND PROTEOME

doi:10.1136/hrt.2010.205781.42

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Rationale We have recently established a transgenic mouse model for conditional induction of long-term hibernation via myocardium-specific induction of a VEGF-sequestering soluble receptor.

Objective Using a combined '-omics' approach, we aim to resolve the cardioprotective response that preserves myocardial viability under chronic hypoxia by integrating mRNA, protein and metabolite changes in unsupervised network analysis.

Methods and results A genome array, difference in gel electrophoresis and proton nuclear magnetic resonance spectroscopy were employed to dissect the hibernation process into an initiation and a maintenance phase. The initiation phase was characterised by peak levels of K(ATP) channel and glucose transporter 1 (GLUT1) expression. Glibenclamide, an inhibitor of K(ATP) channels, blocked GLUT1 induction. In the maintenance phase, tissue hypoxia and GLUT1 expression were reduced and metabolite concentrations were kept relatively constant. Unguided bioinformatics analysis on the combined datasets confirmed that anaerobic glycolysis was affected and that the observed enzymatic changes in cardiac metabolism were directly linked to hypoxia-inducible factor (HIF)-1 activation. Notably, the combination of the proteomic and transcriptomic datasets improved the statistical confidence of the pathway analysis by two orders of magnitude, with HIF—hypoxia—Akt signalling and glycolysis being the most significant.

Conclusions We demonstrate how combining different '-omics' datasets aids in the identification of key biological pathways: chronic hypoxia resulted in a pronounced adaptive response at the transcript and the protein level to keep metabolite levels steady. This preservation of metabolic homoeostasis is likely to contribute to the long-term survival of the hibernating myocardium.

BAS/BSCR32 NICOTINIC ACID ADENINE NUCLEOTIDE PHOSPHATE IS INVOLVED IN ISCHAEMIA-REPERFUSION-INDUCED CA2+ OSCILLATIONS AND CELL DEATH

doi:10.1136/hrt.2010.205781.43

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Reperfusion of ischaemic cells causes intracellular Ca^{2+} oscillations as the sarcoplasmic reticulum (SR) takes up and releases Ca^{2+}, leading to hypercontracture and cell death. In other systems, nicotinic acid adenine nucleotide phosphate (NAADP) acts as a second messenger to stimulate Ca^{2+} release from acidic intracellular Ca^{2+} stores, which in turn triggers Ca^{2+} release from the SR. We hypothesised that NAADP signalling is involved in the Ca^{2+} fluctuations that occur at reperfusion.

We examined the effects of a novel NAADP inhibitor, Ned-19, on ischaemia-reperfusion injury in isolated adult rat ventricular cardiomyocytes (ARVC). The sensitivity of mitochondrial permeability transition pore (mPTP) was measured in ARVC using a laser-induced

oxidative stress model. SR Ca^{2+} release was measured by treating cells loaded with fluorescent dye, fluo-4-AM, with caffeine. Cardioprotection was tested by exposing ARVC to metabolic ischaemia-reperfusion. Ned-19 was found to significantly delay the time to mPTP opening by 76%±16%, 55%±20%, 47%±19% and 44%±17% (all $p<0.05$) at concentrations of 100 μmol/l, 10 μmol/l, 1 μmol/l and 0.1 μmol/l, respectively, compared with the control group. Concentrations of Ned-19 at 100 μmol/l, 10 μmol/l and 1 μmol/l, but not 0.1 μmol/l, significantly inhibited caffeine-stimulated SR Ca^{2+} release (71.6%±2.0%, 34.2%±1.9%, 55.6%±5.5% and −14%± 21%, respectively) indicating non-specific effects at higher concentrations. A low dose of 0.1 μmol/l Ned-19 increased the survival of cells following metabolic ischaemia-reperfusion to 46%±19% from 29% (control).

In conclusion, we have shown the involvement of NAADP in SR Ca^{2+} release and mPTP opening, and that by inhibiting NAADP signalling at reperfusion with Ned-19, cardiomyocytes may be protected against ischaemia-reperfusion injury.

BAS/BSCR33 CARDIOPROTECTION BY HYPOXIA-INDUCIBLE FACTOR-1α: UNDERLYING BENEFICIAL EFFECTS ON MITOCHONDRIAL FUNCTION

doi:10.1136/hrt.2010.205781.44

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Introduction Activation of hypoxia-inducible factor-1α (HIF-1α) protects the heart from ischaemia-reperfusion injury, although the underlying mechanisms are unclear. We hypothesised that HIF-1α-induced cardioprotection is mediated by beneficial effects on mitochondrial function.

Methods and results Two different experimental models of HIF-1α activation were used: (1) pharmacological inhibition of proline hydroxylase (PHD) and (2) genetic inactivation of von Hippel–Lindau (VHL), proteins responsible for HIF-1α degradation under normoxic conditions. A single dose (3 mg/kg) of the PHD inhibitor (GSK0360A or PHDi), administered by oral gavage 4 h before ex vivo myocardial infarction, reduced myocardial infarct size (percentage of the area at risk) in male Sprague–Dawley rats (30.6%±2.9% PHDi vs 44.2%±2.9%; $p<0.5$; $N>5$). Next, conditional cardiac-specific VHL knockout mice (VHL-KO) that express an inducible Cre-recombinase transgene to delete the VHL-floxed gene within the heart following tamoxifen induction, expressed higher levels of HIF-1 in the heart as assessed by immunostaining. The activation of myocardial HIF-1 resulted in a smaller myocardial infarct size in comparison with the littermate control (29.1%±4.7% in VHL-KO vs 52.5%±3.3% in control; $p<0.05$; $N>5$/group). In VHL-KO cardiomyocytes subjected to simulated ischaemia-reperfusion injury (SIRI) (120 min ischaemia and 15 min reperfusion, the production of reactive oxygen species (ROS) (measured by reduced Mitotracker Red fluorescence)(1.0%±0.1-fold increase in VHL-KO vs 1.3%±0.2-fold increase in control; $p<0.05$; $N>3$ experiments each with 40 cells) and mitochondrial permeability transition pore (mPTP) opening sensitivity was reduced (measured by TMRM fluorescence) (1.1%±0.1 fold increase in VHL-KO vs 1.4%±0.1 fold increase in control; $p<0.05$; $N>3$ experiments each with 40 cells).

Conclusions HIF-1α activation by genetic deletion of VHL or pharmacological inhibition of PHD, is cardioprotective and this protective effect can be attributed in part to beneficial effects on the mitochondria.

BAS/BSCR34 IS AN INCREASED AMPK ACTIVATION DURING ISCHAEMIA ESSENTIAL FOR THE PROTECTION OF THE HEART AGAINST INFARCTION?

doi:10.1136/hrt.2010.205781.45

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Background During ischaemia, AMPK activation occurs in order to provide energy from alternative resources. However, AMPK activity is known to be impaired in diabetes. We hypothesised that enhancing AMPK activation above physiological levels during ischaemia would protect both the normoglycaemic and the diabetic heart.

Methodology Hearts from Wistar and Goto Kakizaki rats (GK, a mildly diabetic rat strain) were subjected to 35 min coronary artery occlusion in the presence of 10, 20 or 40 μM A-769662 (an activator of AMPK), followed by 120 min of reperfusion with normal buffer ($n \geq 6$). Risk zone and myocardial infarction were assessed using Evans blue and 2,3,5-triphenyltetrazolium chloride (TTC) staining, respectively and expressed as percentage of the area at risk (I/R%). The effect of A-769662 on mitochondrial permeability transition (opening of the mPT pore is associated with reperfusion injury) was also investigated by exposing rat cardiomyocytes loaded with the fluorophore TMRM to a laser oxidative insult; the time to mitochondrial membrane depolarisation and rigour contracture were measured (n=6, 80–100 cells/assay).

Results A-769662 reduced the infarct size in both the normoglycaemic and diabetic hearts in comparison with control hearts at 20 μM (31.8%±3.1 vs 51.4%±1.5 normoglycaemic heart; 22.7%±3.0 vs 37.6%±2.7 for the GK heart; $p<0.05$) and at 40 μM (35.6%±1.9 vs 51.4%±1.5 for the normoglycaemic heart; 18.6%±1.6 vs 37.6%±2.7, for the GK heart; $p<0.05$) In addition, A-769662 also significantly delayed the mPTP opening (147.71%±10.2% at 20 μM, 146.7%± 15.6% at 40 μM, vs control 100%, $p<0.05$).

Conclusions Our data suggest that the enhancement of AMPK activity during ischaemia may lead to infarct reduction and delayed opening of the mPTP in the ischaemic reperfused rat heart.

BAS/BSCR35 EFFECTS OF ALDOSTERONE AND OBESITY ON THE ANTICONTRACTILE PROPERTIES OF PERIVASCULAR ADIPOSE TISSUE IN RAT AORTIC RINGS

doi:10.1136/hrt.2010.205781.46

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The mechanisms by which perivascular adipose tissue (PVAT) can reduce vascular contractility remain to be elucidated and may underlie the associations of obesity with hypertension, insulin resistance and cardiovascular disease. This study investigates the effects of aldosterone and obesity in isolated rat aorta. Healthy and obese male rats were killed by stunning and cervical dislocation. The mesenteric bed was removed and arteries dissected with and without PVAT. Arteries were mounted on a wire myograph and were constricted with 60 mM KPSS. Cumulative concentration responses (10^{-9}–10^{-5} M) to norepinephrine (NE) were performed before and after 10 min incubation with aldosterone (5 nM). Endothelial integrity was confirmed by relaxation to 10^{-5} M acetylcholine. Responses are expressed as mean (±SEM) percentage of KPSS constriction and analysed using two-way ANOVA. PVAT (n=10) significantly ($p<0.05$) reduced constriction in healthy vessels