BAS/BSCR26 HISTONE DEACETYLASE 3 PROTECTS ENDOTHELIAL CELLS FROM INFLAMMATION VIA REGULATION OF GALECTIN 9 EXPRESSION

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Histone deacetylase 3 (HDAC3), a member of the class I histone deacetylases, is known to have a crucial role in endothelial cell differentiation[1 2] and maintenance of endothelial integrity in response to disturbed flow.[3] In this study, we investigated the function of HDAC3 in endothelial protection from inflammation, and the underlying mechanism. The inflammatory mediator lipopolysaccharides (LPS) induced HDAC3 and galectin-9 production in a similar pattern in human umbilical vein endothelial cells. Overexpression of HDAC3 by adenoviral gene transfer increased galectin-9 expression. Pharmacological inhibition of HDAC activity with a pan-HDAC inhibitor (TSA) or HDAC3 specific inhibitor (apicidin) reduced the baseline and LPS-induced galectin-9 expression. In addition, knockdown of HDAC3 through shRNA lentiviral transfection abolished the baseline and LPS-induced galectin-9 expression. Similar results were observed on interferon γ (IFNγ)-induced galectin-9 expression. To explore the underlying mechanism, the interaction of HDAC3 with galectin-9 upstream signal pathway phosphoinositol-3-kinase (PI3K)/signal transmission and transducer 3 (Stat3)/interferon response factor (IRF3) was assessed. Co-immunoprecipitation assay showed that HDAC3 formed a complex with PI3K/Stat3/IRF3, which was enhanced by LPS and IFNγ treatment. Using truncated forms of HDAC3, it was shown that the C-terminal of HDAC3 was responsible for the formation of the complex. Furthermore, venous administration of LPS or IFNγ to mice increased HDAC3 expression and binding to the above-mentioned proteins, leading to galectin-9 expression in the aorta. These results suggest that HDAC3 may protect endothelial cell from inflammation through galectin-9 expression, which may have an impact on preventing vascular inflammation related to the development of atherosclerosis.

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BAS/BSCR27 DIESEL EXHAUST PARTICLES PROMOTE ATHEROSCLEROSIS IN APOLIPOPROTEIN E-DEFICIENT MICE

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Air pollution has been linked to the development of atherosclerosis and cardiovascular disease. Diesel exhaust particulate (DEP) accounts for a substantial proportion of urban air pollution but its effects on atherogenesis are unknown. We hypothesised that DEP will exacerbate plaque formation in a murine model of atherosclerosis.

Apolipoprotein E knockout (ApoE) mice (10–12 weeks; n=16) were fed a 'Western diet' (21% cholesterol) for 8 weeks to induce the development of 'complex' atherosclerotic plaques. During the last 4 weeks of feeding, mice underwent twice-weekly intratracheal instillation of 35 µl DEP (1 mg/ml; National Institute of Standards and Technology) or vehicle (saline).

Histological sections of the brachiocephalic artery from ApoE knockout mice showed large, foam cell-filled fibrous plaques. Plaque burden was increased (p=0.025; n=5–6) in DEP-treated mice (69±9%) compared with vehicle-treated controls (42±7%). Furthermore, plaques from DEP-treated mice exhibited a greater number of adjoining (2.3±0.2%) and buried (1.2±0.3%) fibrous caps than control mice (1.7±0.2% and 0.2±0.1%, respectively; $p<0.05$ n=5). There was no evidence of systemic inflammation, increased circulating blood lipids or endothelial dysfunction in DEP-treated animals.

This is the first study to show that pulmonary exposure to the particulate matter within diesel exhaust enhances atherogenesis. This action may, therefore, contribute to the increased cardiovascular morbidity and mortality associated with air pollution. This model will allow identification of the constituents of DEP that mediate this atherogenic effect and provide an important insight into potential interventions to reduce the impact of vehicular emissions.

BAS/BSCR28 BLOCKADE OF ADENOSINE A2A RECEPTOR ATTENUATES ANGIOTENSIN II-INDUCED REACTIVE OXYGEN SPECIES PRODUCTION AND IMPAIRMENT OF ENDOTHELIUM-DEPENDENT VESSEL RELAXATION IN MOUSE AORTAS

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The endothelium expresses abundantly an adenosine A2A receptor (A2AR) which has important roles in the regulation of vascular function. The endothelium expresses also the AT1-type receptor for angiotensin II (AngII) which has been found to activate a reactive oxygen species (ROS)-generating NADPH oxidase and cause oxidative damage to the endothelium. However, little is known about the role of A2AR in AngII-induced endothelial ROS production. In this study, we investigated the effect of A2AR blockade on AngII-induced ROS production and endothelium function using freshly isolated aortas from CD1 mice at 10–12 weeks of age. Compared with vessels treated with vehicle, acute AngII (200 nM for 45 min) treatment significantly increased ROS production in the vessel wall as detected by DHE fluorescence and this was accompanied by increased ERK1/2 phosphorylation. These AngII effects were inhibited, returning to control levels in the presence of a specific A2AR antagonist, SCH58261 (100 nM). Compared with control vessels, treatment with AngII severely compromised the endothelium-dependent vessel relaxation to acetylcholine as assessed by an organ bath. Addition of SCH58261 (100 nM) or tiron (20 mM, a specific cell membrane permeable superoxide scavenger) during AngII stimulation protected the endothelium from AngII damage and preserved endothelium-dependent vessel relaxation to acetylcholine. The endothelium dependence of the relaxation to acetylcholine was confirmed by mechanical denudation of the endothelium. In conclusion, blockade of A2AR protects the endothelium from acute AngII-induced oxidative stress, ERK1/2 phosphorylation and endothelium dysfunction.

BAS/BSCR29 APOLIPOPROTEIN(A) IMPAIRS ADAPTIVE REMODELLING IN HUMAN SAPHENOUS VEIN ENDOTHELIAL AND SMOOTH MUSCLE CELLS

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Apolipoprotein(a) (apo(a)), a unique glycoprotein component of plasma lipoprotein(a) (Lp(a)),exhibits resistance to classical

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

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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

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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

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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