BAS/BSCR YIA abstract

YIA1 INTERACTION BETWEEN HDAC3 AND XBP1 IS CRITICAL IN MAINTAINENCE OF ENDOTHELIAL INTEGRITY

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Histone deacetylases (HDACs) play a crucial role in transcriptional regulation through modulation of chromatin structure. The class I HDAC, HDAC3, is involved in maintaining endothelial cell integrity.[1] Sustained activation of the x-box binding protein 1 (XBP1), an endoplasmic reticulum stress response transcription factor, results in the development of atherosclerosis in apoE−/− mice.[2] HDAC3 and XBP1 are similarly expressed in the bifurcation regions of the aorta. In this study, we investigated whether cross-talk existed between HDAC3 and XBP1, and its role in the maintenance of endothelial cell integrity. Our study demonstrated that disturbed flow upregulated HDAC3 and unspliced XBP1 (XBP1u) protein production through the KDR/PI3K/Akt pathway. Knockdown of XBP1 by shRNA lentiviral transfection ablated disturbed flow-induced HDAC3 upregulation. Similarly to HDAC3, overexpression of XBP1u by adenoviral gene transfer increased Akt phosphorylation at Serine473 and haem oxygenase 1 gene transcription, which showed a protective role in hydrogen peroxide-induced apoptosis of endothelial cells. Co-immunoprecipitation assays demonstrated that HDAC3 physically associates with XBP1u and this could be enhanced by disturbed flow and VEGF treatment. The use of truncated HDAC3 constructs demonstrated that XBP1 binds to the central section of HDAC3. Further experiments indicated that overexpression of XBP1u increased the binding of HDAC3 to IRE1α, Akt and PI3K, especially after VEGF treatment. In contrast, sustained activation of spliced XBP1 decreased HDAC3 protein production through transcriptional suppression, leading to endothelial apoptosis. These results suggest that XBP1u protects endothelial cells from oxidative stress by interacting with HDAC3. Targeting this interaction may provide novel therapeutic strategies for vascular disease via maintaining endothelial integrity.

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YIA2 11β-HSD1 DEFICIENCY ATTENUATES ATHEROSCLEROSIS IN APOE−/− MICE: ROLE OF BOTH GLUCOCORTICOID AND NON-GLUCOCORTICOID (OXYSTEROL) FACTORS

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11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) regenerates active glucocorticoids, amplifying intracellular actions.[1] 11β-HSD1 deficiency or inhibition improves metabolic syndrome and attenuates atherosclerosis in vulnerable rodent strains and is a target for drug development.[2–4] However, 11β-HSD1 also catalyses conversion of 7-ketocholesterol,[5] which accumulates in fatty tissues,[6] to potentially more atherogenic 7β-hydroxycholesterol. Whether atheroprotection with 11β-HSD1 deficiency is dependent on glucocorticoid or oxysterol effects is unknown. Male atherosclerosis-prone ApoE−/− and ApoE−/−.11β-HSD1−/− double knockout (DKO) mice underwent adrenalectomy or sham surgery (n=8/group), then received a high (0.2%) cholesterol Western diet for 12 weeks. The aorta and branches were perfusion-fixed. Lesion volume and extracellular lipids were determined by 3D optical projection tomography. Data are mean±SE of the means. Adrenalectomy had no effect on body/organ weights in either genotype. Removal of endogenous glucocorticoids by adrenalectomy in ApoE−/− mice did not reduce lesion volume (232±24 vs 235±34 μm^3 sham control). DKO mice had reduced lesion volumes (139±17 μm^3) compared with ApoE−/−($p<0.05$). Adrenalectomy reversed this effect (263±52 μm^3).

Adrenalectomised DKO mice had increased extracellular lipids (73.6±2.6 μm^3) within the lesion compared with either ApoE−/− adrenalectomised (37.4±5.2 μm^3), ApoE−/− sham (42.9±5.5 μm^3) or DKO sham (44.2±12 μm^3) group. Thus circulating glucocorticoids are necessary for 11β-HSD1 deficiency to attenuate atherosclerosis. However, 11β-HSD1 deficiency increases the lipid content of plaques in the absence of glucocorticoids, perhaps owing to accumulation of 7-ketocholesterol? Consequently, both reactions of 11β-HSD1 may be involved in the effects of the enzyme on atherogenesis.

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YIA3 PROTEOMIC ANALYSIS OF THE CARDIAC MYOFILAMENT SUBPROTEOME REVEALS DYNAMIC ALTERATIONS IN PHOSPHATASE SUBUNIT DISTRIBUTION

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Rationale Myofilament proteins are responsible for cardiac contraction. The myofilament subproteome, however, has not been comprehensively analysed thus far.

Methods Cardiomyocytes were isolated from rodent hearts and stimulated with endothelin-1 and isoproterenol, potent inducers of myofilament protein phosphorylation. Subsequently, cardiomyocytes were 'skinned' and the myofilament subproteome analysed using a high mass accuracy ion trap tandem mass spectrometer (LTQ Orbitrap XL) equipped with electron transfer dissociation.

Results As expected, a small number of myofilament proteins constituted the majority of the total protein mass, with several known phosphorylation sites being confirmed by electron transfer dissociation. More than 600 additional proteins were identified in the cardiac myofilament subproteome, including kinases and phosphatase subunits. The proteomic comparison of myofilaments from control and treated cardiomyocytes suggested that isoproterenol treatment altered the subcellular localisation of protein phosphatase 2A regulatory subunit B56α. Immunoblot analysis of myocyte

fractions confirmed that β-adrenergic stimulation by isoproterenol decreased the B56α content of the myofilament fraction in the absence of significant changes in the myosin phosphatase target subunit isoforms 1 and 2 (MYPT1 and MYPT2). Furthermore, immunolabelling and confocal microscopy revealed the spatial redistribution of these proteins, with a loss of B56α from Z-disc and M-band regions but increased association of MYPT1/2 with A-band regions of the sarcomere, following β-adrenergic stimulation.

Conclusion We present the first comprehensive proteomic dataset of skinned cardiomyocytes and demonstrate the potential of proteomics to unravel dynamic changes in protein composition that may contribute to the neurohormonal regulation of myofilament contraction.

YIA4 C JUN N-TERMINAL KINASE PROMOTES ENDOTHELIAL ACTIVATION AT ATHEROSCLEROSIS-SUSCEPTIBLE SITES BY ENHANCING EXPRESSION OF NF-κB TRANSCRIPTION FACTORS

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Atherosclerosis develops predominantly at branches and bends in arteries that are exposed to disturbed flow which exerts low, oscillatory shear stress on endothelial cells (ECs). We demonstrated that c-Jun N-terminal kinase (JNK) is activated in ECs at atherosusceptible but not atheroprotected sites. Transcriptome profiling of cultured ECs treated with a pharmacological inhibitor revealed that JNK functions as a positive regulator of NF-κB transcription factors, which promote inflammation by inducing inflammatory molecules (eg, VCAM-1). This observation was confirmed by silencing of JNK1 and ATF2 (a downstream transcription factor), which led to reduced NF-κB expression in cultured ECs. We validated our findings by demonstrating that EC expression of NF-κB and VCAM-1 and the accumulation of CD68-positive macrophages was elevated at atherosusceptible sites compared with atheroprotected sites in aortas of wild-type mice. Genetic deletion of JNK1 suppressed NF-κB and VCAM-1 expression, and reduced macrophage accumulation at the atherosusceptible site, indicating that JNK1 positively regulates NF-κB expression and inflammation. To establish a causal relationship between shear stress and JNK activity, we altered blood flow in the murine carotid artery by placing a constrictive cuff. We observed that low, oscillatory shear stress can enhance JNK activity, NF-κB and VCAM-1 expression in ECs and promote macrophage accumulation in arteries. We conclude that JNK1-ATF2 signalling promotes EC activation and inflammation at atheroprone sites exposed to low, oscillatory shear stress by enhancing NF-κB expression. Our findings illuminate a novel level of cross-talk between the NF-κB and JNK signalling pathways that may influence the spatial distribution of atherosclerotic lesions.

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YIA5 PKCδ DEFICIENCY ACCELERATES NEOINTIMAL LESIONS IN A MOUSE MODEL OF VASCULAR INJURY INVOLVING DELAYED RE-ENDOTHELIALISATION AND VASOHIBIN-1 ACCUMULATION

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Objective Protein kinase C (PKC) δ functions as a signal transducer mediating several essential functions of cell proliferation and apoptosis. However, the effect of PKCδ on neointimal formation is currently unknown. In this study, we used a vascular injury model in PKCδ knockout mice to investigate the role of PKCδ in the lesion development and underlying mechanism.

Methods and results 3 weeks after wire injury of femoral arteries, neointimal lesions were significantly increased in PKCδ−/− mice compared with wild-type animals. Immunohistochemical staining revealed that total numbers of smooth muscle cells and macrophages in the lesions were markedly elevated without the alterations of the ratio between these two types of cells. To further study the mechanisms of PKCδ-mediated increase in the lesion, an in vivo endothelial migration model was established to evaluate endothelial wound healing after wire injury. Data indicate that re-endothelialisation of the injured vessel was markedly delayed in PKCδ−/− mice that coincided with more severe intimal hyperplasia. When endothelial cells were cultivated from cardiac tissues of PKCδ−/− and PKCδ+/+ mice, the ability of cell migration was significantly reduced in PKCδ−/− mice, but no difference in proliferation and apoptosis was found. This altered endothelial migration was also verified by PKCδ inhibitor and siRNA techniques in wild-type cells. Interestingly, vasohibin-1, an anti-migration protein, was elevated in endothelial cells derived from PKCδ-deficient mice, which was identified largely owing to delayed protein degradation mediated by PKCδ. Downregulation of vasohibin-1 restored the migration rate of PKCδ−/− endothelial cells to a level similar to that in wild-type cells.

Conclusion Our data provide the first evidence that PKCδ-enhanced neointima formation occurs mainly due to delayed re-endothelialisation, which is mediated by increased cellular vasohibin-1 that is regulated by PKCδ.

YIA6 EFFECTS OF GLP-1 ELUTING STEM CELL THERAPY ON COLLAGEN REMODELLING, INFARCT SIZE AND APOPTOSIS IN A PORCINE MODEL OF MYOCARDIAL INFARCTION

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Glucagon-like peptide-1 (GLP-1) is a gut incretin hormone with cardioprotective effects. Human stem cells secreting a GLP-1 fusion protein and encapsulated in an alginate matrix (GLP-1 CellBeads) have been developed as a novel therapeutic agent. This study investigated the effects of GLP-1 CellBeads on post-myocardial infarction (MI) healing in a porcine model. GLP-1 CellBeads were delivered to the left anterior descending coronary artery to create micro-infarcts, with cell-free beads as controls. Hearts were explanted at 1 and 4 weeks post-MI. Gross infarct size was measured as a percentage of left ventricular area. Tissue was analysed for inflammation (number of MAC 387 positive cells/mm^2), apoptosis (% TUNEL positive cells) and collagen (% picrosirius red staining).

Compared with controls (n=4), the GLP-1-treated group (n=6) exhibited less infarct at one (6.21%±0.64 vs 9.78%±1.80% 2LV, p=NS) and 4 weeks post-MI (4.7%±2.1 vs 21.8%±4.8% LV, p=0.02). Within the infarct there was increased inflammation in GLP-1-treated groups at both time points (1 week: 97.22%±19.62 vs 36.67%±7.78, p=0.01; 4 weeks: 24.2%±4.57 vs 12.3%±3.16, p=0.03). At 1 week, apoptosis rates in the infarct area were similar in both groups (1.46 %±0.57 vs 1.47 %±0.17, p=NS), with less apoptosis in the GLP-1-treated group at 4 weeks (0.51%±0.18 vs 1.84%±0.62, p=NS). Collagen content at 1 week was lower (5.14%±1.19 vs 9.95%±1.42, p=0.05) and at 4 weeks higher in the treated group (20.89%±8.25 vs 6.87%±2.92, p=NS). GLP-1 CellBeads have an effect on post-MI infarct size, inflammation and