BAS/BSCR YIA abstract

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

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D Martin, H Li, A Zampetaki, Y Hu, L Zeng, Q Xu. *Cardiovascular Division, King's College London BHF Centre, London, UK*

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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T Mitic, P W F Hadoke, S Chuaiphichai, T Y Man, E Miller, R Andrew, B R Walker, K E Chapman, J R Seckl. *Endocrinology Unit, Centre for Cardiovascular Sciences, The Queen's Medical Research Institute, University of Edinburgh, Scotland, UK*

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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[1]X Yin, [1]F Cuello, [1]U Mayr, [2]M Hornshaw, [1]E Ehler, [1]M Avkiran, [1]M Mayr. [1]*King's BHF Centre, King's College London, London, UK;* [2]*ThermoFisher Scientific, Hemel Hempstead, UK*

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

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